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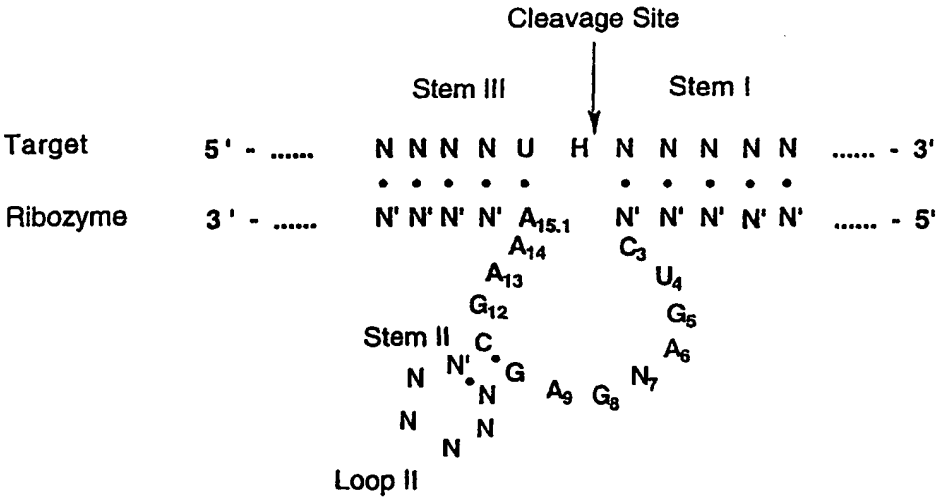
(54) Title: COMPOSITIONS AND METHOD FOR MODULATION OF ALKALOID BIOSYNTHESIS AND FLOWER FORMATION IN PLANTS



(57) Abstract

An enzymatic nucleic acid molecule with RNA cleaving activity, wherein the nucleic acid molecule modulates the expression of a gene involved in the biosynthesis of alkaloid compounds and flower formation in a plant. A transgenic plant comprising nucleic acids encoding for an enzymatic nucleic acid molecule with RNA cleaving activity, wherein the nucleic acid molecule modulates the expression of a gene involved in the biosynthesis of alkaloid compounds of flower formation in a plant. An enzymatic nucleic acid molecule with RNA cleaving activity, wherein the nucleic acid molecule modulates the expression of solanidine UDP-glucose glucosyl-transferase gene or citrate synthase in plants.

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DESCRIPTION

Compositions And Method For Modulation Of Alkaloid Biosynthesis And Flower Formation In Plants

Background of the Invention

The present invention concerns compositions and methods for the modulation of gene expression in plants, specifically using enzymatic nucleic acid molecules.

The following is a brief description of regulation of gene expression in plants. The discussion is not meant to be complete and is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

There are a variety of strategies for modulating gene expression in plants. Traditionally, antisense RNA (reviewed in Bourque, 1995 Plant Sci 105, 125-149) and co-suppression (reviewed in Jorgensen, 1995 Science 268, 686-691) approaches have been used to modulate gene expression. Insertion mutagenesis of genes have also been used to silence gene expression. This approach, however, cannot be designed to specifically inactivate the gene of interest. Applicant believes that ribozyme technology offers an attractive new means to alter gene expression in plants.

Naturally occurring antisense RNA was first discovered in bacteria over a decade ago (Simons and Kleckner, 1983 Cell 34, 683-691). It is thought to be one way in which bacteria can regulate their gene expression (Green et al., 1986 Ann. Rev. Biochem. 55: 567-597; Simons 1988 Gene 72: 35-44). The first demonstration of antisense-mediated inhibition of gene expression was reported in mammalian cells (Izant and Weintraub

1984 Cell 36: 1007-1015). There are many examples in the literature for the use of antisense RNA to modulate gene expression in plants. Following are a few examples:

Shewmaker et al., U.S. Patent Nos. 5,107,065 and 5, 453,566 disclose methods for regulating gene expression in plants using antisense RNA.

It has been shown that an antisense gene expressed in plants can act as a dominant suppressor gene. Transgenic potato plants have been produced which express RNA antisense to potato or cassava granule bound starch synthase (GBSS). In both of these cases, transgenic plants have been constructed which have reduced or no GBSS activity or protein. These transgenic plants give rise to potatoes containing starch with dramatically reduced amylose levels (Visser et al. 1991, Mol. Gen. Genet. 225: 2889-296; Salehuzzaman et al. 1993, Plant Mol. Biol. 23: 947-962).

Kull et al., 1995, J. Genet. & Breed. 49, 69-76 reported inhibition of amylose biosynthesis in tubers from transgenic potato lines mediated by the expression of antisense sequences of the gene for granule-bound starch synthase (GBSS). The authors, however, indicated a failure to see any in vivo activity of ribozymes targeted against the GBSS RNA.

Antisense RNA constructs targeted against Δ-9 desaturase enzyme in canola have been shown to increase the level of stearic acid (C18:0) from 2% to 40% (Knutzon et. al., 1992 Proc. Natl. Acad. Sci. 89, 2624). There was no decrease in total oil content or germination efficiency in one of the high stearate lines. Several recent reviews are available which illustrate the utility of plants with modified oil composition (Ohlrogge, J. B. 1994 Plant Physiol. 104, 821; Kinney, A. J. 1994 Curr.

Opin. Cell Biol. 5, 144; Gibson et al. 1994 Plant Cell Envir. 17, 627).

Homologous transgene inactivation was first documented in plants as an unexpected result of inserting a transgene in the sense orientation and finding that both the gene and the transgene were down-regulated (Napoli et al., 1990 Plant Cell 2: 279-289). There appears to be at least two mechanisms for inactivation of homologous genetic sequences. One appears to be transcriptional inactivation via methylation, where duplicated DNA regions signal endogenous mechanisms for gene silencing. This approach of gene modulation involves either the introduction of multiple copies of transgenes or transformation of plants with transgenes with homology to the gene of interest (Ronchi et al 1995 EMBO J. 14: 5318-5328). The other mechanism of co-suppression is post-transcriptional, where the combined levels of expression from both the gene and the transgene is thought to produce high levels of transcript which triggers threshold-induced degradation of both messages (van Bokland et al., 1994 Plant J. 6: 861-877). The exact molecular basis for co-suppression is unknown.

Unfortunately, both antisense and co-suppression technologies are subject to problems in heritability of the desired trait (Finnegan and McElroy 1994 Bio/Technology 12: 883-888). Currently, there is no easy way to specifically inactivate a gene of interest at the DNA level in plants (Pazkowski et al., 1988 EMBO J. 7: 4021-4026). Transposon mutagenesis is inefficient and not a stable event, while chemical mutagenesis is highly non-specific.

Applicant believes that ribozymes present an attractive alternative and because of their catalytic mechanism of action, have advantages over competing

technologies. However, there have been difficulties in demonstrating the effectiveness of ribozymes in modulating gene expression in plant systems (Mazzolini et al., 1992 Plant Mol. Biol. 20: 715-731; Kull et al., 1995 J. Genet. & Breed. 49: 69-76). Although there are reports in the literature of ribozyme activity in plants cells, almost all of them involve down regulation of exogenously introduced genes, such as reporter genes in transient assays (Steinecke et al., 1992 EMBO J. 11:1525-1530; Perriman et al., 1993 Antisense Res. Dev. 3: 253-263; Perriman et al., 1995, Proc. Natl. Acad. Sci. USA, 92, 6165).

There are also several publications, [e.g., Lamb and Hay, 1990, J. Gen. Virol. 71, 2257-2264; Gerlach et al., International PCT Publication No. WO 91/13994; Xu et al., 1992, Science in China (Ser. B) 35, 1434-1443; Edington and Nelson, 1992, in Gene Regulation: Biology of antisense RNA and DNA, eds. R. P. Erickson and J. G. Izant, pp 209-221, Raven Press, NY.; Atkins et al., International PCT Publication No. WO 94/00012; Lenee et al., International PCT Publication Nos. WO 94/19476 and WO 9503404, Atkins et al., 1995, J. Gen. Virol. 76, 1781-1790; Gruber et al., 1994, J. Cell. Biochem. Suppl. 18A, 110 (X1-406) and Feyter et al., 1996, Mol. Gen. Genet. 250, 329-338], that propose using hammerhead ribozymes to modulate: virus replication, expression of viral genes and/or reporter genes. None of these publications report the use of ribozymes to modulate the expression of plant genes.

Mazzolini et al., 1992, Plant. Mol. Bio. 20, 715-731; Steinecke et al., 1992, EMBO. J. 11, 1525-1530; Perriman et al., 1995, Proc. Natl. Acad. Sci. USA., 92, 6175-6179; Wegener et al., 1994, Mol. Gen. Genet. 245, 465-470; and Steinecke et al., 1994, Gene, 149, 47-54,

describe the use of hammerhead ribozymes to inhibit expression of reporter genes in plant cells.

Bennett and Cullimore, 1992 Nucleic Acids Res. 20, 831-837 demonstrate hammerhead ribozyme-mediated in vitro cleavage of glna, glnb, glng and glnd RNA, coding for glutamine synthetase enzyme in Phaseolus vulgaris.

Certain plants contain undesirable alkaloid compounds which, when present in excess, are undesirable for human or animal consumption (Valkonen et al. 1996 Crit. Rev. Plant Sci. 15, 1-20). Potatoes and other solanaceous plants contain steroidal glycoalkaloids, whose level is regulated by genetic, developmental and environmental signals (Bergenstrahle et al. 1992 J. Plant Phys. 140, 269-275; Sinden, 1984 Am. Potato J. 61, 141-156). Potato tubers synthesize the alkaloids solanine and chaconine in response to wounding, temperature, light and sprouting. These glycoalkaloids are thought to be responsible for preventing insect predation and resistance to infection by pathogenic fungi (Valkonen et al. supra). The enzyme solanidine UDP-glucose glucosyltransferase is implicated as the enzyme primarily responsible for the biosynthesis of both these alkaloid compounds (Stapleton et al. 1992 Prot. Exp. Purif. 3, 85-92, 6; Stapleton et al. 1991 J. Agri. Food Chem. 39, 1187-1193).

The mitochondrial tricarboxylic acid (TCA) cycle enzyme citrate synthase is implicated in the formation of flower buds in plants (Landshutze et al., 1995 EMBO J. 14, 660-666). Experiments with antisense constructs have shown that inhibition of the expression of the gene for this enzyme can delay or eliminate flower bud formation. There were no visible effects on plant growth or yield. The ovaries in the transgenic antisense plants disintegrated, indicating that citrate synthase and the

TCA cycle are important in the transition from vegetative to generative phase of plant growth. Cytoplasmic male sterility (CMS) has been associated with mitochondrial gene expression, but typically affects the ability of the plant to produce viable pollen, not affecting female fertility (Levings et al., 1993 Plant Cell 5, 1285-1290; Chaudhury, 1993 Plant Cell 5, 1277-1283). Inhibition of expression of the citrate synthase gene by ribozymes should result in the delay or elimination of flower formation in plants. This would be very useful in preventing flowering in plant species that are vegetatively propagated or where the primary consumable part of the plant is root, stem or leaf. The enzyme is mitochondrial, but is encoded by a nuclear gene (Landshutze et al.,1995 Planta 196, 756-764). Chemical inhibition of mitochondrial respiration is harmful (Kromer et al.,1991 Plant. Phys. 95, 1270-1276), thus the ribozyme genetic approach is potentially advantageous over other methods.

The references cited above are distinct from the presently claimed invention since they do not disclose and/or contemplate the use of ribozymes to down regulate genes involved in the plant alkaloid biosynthesis in plant cells, let alone plants.

Summary Of The Invention

The invention features modulation of gene expression in plants specifically using enzymatic nucleic acid molecules. Preferably, invention features inhibiting the expression of genes involved in the biosynthesis of certain alkaloid compounds using enzymatic nucleic acid molecules. That is, the inhibition of the gene product (e.g., RNA) results in a lowering of the production of alkaloid in the plant. Limiting the levels of certain

alkaloid compounds in commercial cultivars, especially reductions in alkaloid content in the tuber by use of tissue-specific promoters is disclosed. The isolation of the gene encoding solanidine glucosyltransferase now allows evaluation of the phenotype that results from down-regulation of this gene (Moehs et al., 1997 Plant J. 11, 100-110). This application further deals with methods to produce cultivars such as, potato, tomato, pepper, eggplant, ditura, and others, with low levels of the toxic alkaloids.

In another aspect, the invention features inhibiting the expression of genes involved in flower formation using enzymatic nucleic acid molecules. That is, the gene product (e.g., RNA) is inhibited to prevent formation of a flower by the plant modulating the expression of citrate synthase in commercial cultivars by use of enzymatic nucleic acid is disclosed as one example. Inhibition of expression of the citrate synthase gene by ribozymes may result in the delay or elimination of flower formation in plants. This would be very useful in preventing flowering in plant species that are vegetatively propagated or where the primary consumable part of the plant is root, stem or leaf. This application further deals with methods to produce cultivars such as, lettuce, spinach, cabbage, brussel sprouts, arugula, kale, collards, chard, beet, turnip, potato, sweet potato and turfgrass, with delayed or elimination of flower formation. Any gene in the flower formation pathway that does not effect vegetative growth can be targeted in this manner.

The enzymatic nucleic acid molecule with RNA cleaving activity may be in the form of, but not limited to, a hammerhead, hairpin, hepatitis delta virus, group I intron, group II intron, RNaseP RNA, Neurospora VS RNA

and the like. The enzymatic nucleic acid molecule with RNA cleaving activity may be encoded as a monomer or a multimer, preferably a multimer. The nucleic acids encoding for the enzymatic nucleic acid molecule with RNA cleaving activity may be operably linked to an open reading frame. Gene expression in any plant species may be modified by transformation of the plant with the nucleic acid encoding the enzymatic nucleic acid molecules with RNA cleaving activity. There are also numerous technologies for transforming a plant: such technologies include but are not limited to transformation with Agrobacterium, bombarding with DNA coated microprojectiles, whiskers, or electroporation. Any target gene may be modified with the nucleic acids encoding the enzymatic nucleic acid molecules with RNA cleaving activity.

Ribozymes can be used to modulate flower formation of a plant, for example, by modulating the activity of an enzyme involved in a biochemical pathway. It may be desirable, in some instances, to decrease the level of expression of a particular gene, rather than shutting down expression completely: ribozymes can be used to achieve this. Enzymatic nucleic acid-based techniques were developed herein to allow directed modulation of gene expression to generate plant cells, plant tissues or plants with altered flowering phenotype.

In a preferred embodiment the invention features Ribozymes that can be used to modulate a specific trait of a plant cell, for example, by modulating the activity of an enzyme involved in a biochemical pathway. It may be desirable, in some instances, to decrease the level of expression of a particular gene, rather than shutting down expression completely: ribozymes can be used to achieve this. Enzymatic nucleic acid-based techniques

were developed herein to allow directed modulation of gene expression to generate plant cells, plant tissues or plants with altered phenotype.

Ribozymes (i.e., enzymatic nucleic acids) are nucleic acid molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence-specific manner. Such enzymatic RNA molecules can be targeted to virtually any RNA transcript, and efficient cleavage has been achieved in vitro and in vivo (Zaug et al., 1986, Nature 324, 429; Kim et al., 1987, Proc. Natl. Acad. Sci. USA 84, 8788; Dreyfus, 1988, Einstein Quarterly J. Bio. Med., 6, 92; Haseloff and Gerlach, 1988, Nature 334 585; Cech, 1988, JAMA 260, 3030; Murphy and Cech, 1989, Proc. Natl. Acad. Sci. USA., 86, 9218; Jefferies et al., 1989, Nucleic Acids Research 17, 1371).

Because of their sequence-specificity, trans-cleaving ribozymes may be used as efficient tools to modulate gene expression in a variety of organisms including plants, animals and humans (Bennett et al., supra; Edington et al., supra; Usman & McSwiggen, 1995 Ann. Rep. Med. Chem. 30, 285-294; Christoffersen and Marr, 1995 J. Med. Chem. 38, 2023-2037). Ribozymes can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the mRNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a particular phenotype and/or disease state can be selectively inhibited.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Figures

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pairs long. Each N is any nucleotide and each • represents a base pair.

Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, Nature, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, Nature, 334, 585-591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, Nucl. Acids. Res., 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (i.e., n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3 - 20 bases, i.e., m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (i.e., r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (e.g., 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (i.e., o and p is each independently from 0 to any number, e.g., 20) as long as some base-pairing is maintained. Essential bases are

shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, i.e., without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "_____" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis Δ virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Detailed Description Of The Invention

The present invention concerns compositions and methods for the modulation of gene expression in plants specifically using enzymatic nucleic acid molecules.

The following phrases and terms are defined below:

By "inhibit" or "modulate" is meant that the activity of enzymes, such as solanidine UDP-glucose glucosyl-transferase, potato citrate synthase, or level of mRNAs encoded by these genes is reduced below that observed in the absence of an enzymatic nucleic acid and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave that target. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA (or DNA) and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. The nucleic acids may be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, nucleozyme, DNAzyme, RNA enzyme, RNAzyme, polyribozymes, molecular scissors, self-splicing RNA, self-cleaving RNA, cis-cleaving RNA, autolytic RNA, endoribonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All of these terminologies describe nucleic acid molecules with enzymatic activity. The term encompasses enzymatic RNA molecule which include one or more ribonucleotides and may include a majority of other types of nucleotides or abasic moieties, as described below.

By "complementarity" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequences by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver and/or express a desired nucleic acid.

By "gene" is meant a nucleic acid that encodes an RNA.

By "plant gene" is meant a gene encoded by a plant.

By "endogenous" gene is meant a gene normally found in a plant cell in its natural location in the genome.

By "foreign" or "heterologous" gene is meant a gene not normally found in the host plant cell, but that is introduced by standard gene transfer techniques.

By "nucleic acid" is meant a molecule which can be single-stranded or double-stranded, composed of nucleotides containing a sugar, a phosphate and either a purine or pyrimidine base which may be same or different, and may be modified or unmodified.

By "genome" is meant genetic material contained in each cell of an organism and/or a virus.

By "mRNA" is meant RNA that can be translated into protein by a cell.

By "cDNA" is meant DNA that is complementary to and derived from a mRNA.

By "dsDNA" is meant a double stranded cDNA.

By "sense" RNA is meant RNA transcript that comprises the mRNA sequence.

By "antisense RNA" is meant an RNA transcript that comprises sequences complementary to all or part of a target RNA and/or mRNA and that blocks the expression of a target gene by interfering with the processing, transport and/or translation of its primary transcript and/or mRNA. The complementarity may exist with any part of the target RNA, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. Antisense RNA is normally a mirror image of the sense RNA.

By "expression", as used herein, is meant the transcription and stable accumulation of the enzymatic

nucleic acid molecules, mRNA and/or the antisense RNA inside a plant cell. Expression of genes involves transcription of the gene and translation of the mRNA into precursor or mature proteins.

By "cosuppression" is meant the expression of a foreign gene, which has substantial homology to an gene, and in a plant cell causes the reduction in activity ~~in~~ of the foreign and/or the endogenous protein product.

By "altered levels" is meant the level of production of a gene product in a transgenic organism is different from that of a normal or non-transgenic organism.

By "promoter" is meant nucleotide sequence element within a gene which controls the expression of that gene. Promoter sequence provides the recognition for RNA polymerase and other transcription factors required for efficient transcription. Promoters from a variety of sources can be used efficiently in plant cells to express ribozymes. For example, promoters of bacterial origin, such as the octopine synthetase promoter, the nopaline synthase promoter, the manopine synthetase promoter; promoters of viral origin, such as the cauliflower mosaic virus (35S); plant promoters, such as the ribulose-1,6-biphosphate (RUBP) carboxylase small subunit (ssu), the beta-conglycinin promoter, the phaseolin promoter, the ADH promoter, heat-shock promoters, and tissue specific promoters. Promoter may also contain certain enhancer sequence elements that may improve the transcription efficiency.

By "enhancer" is meant nucleotide sequence element which can stimulate promoter activity (Adh).

By "constitutive promoter" is meant promoter element that directs continuous gene expression in all cells types and at all times (actin, ubiquitin, CaMV 35S).

By "tissue-specific" promoter is meant promoter element responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (zein, oleosin, napin, ACP).

By "development-specific" promoter is meant promoter element responsible for gene expression at specific plant developmental stage, such as in early or late embryogenesis.

By "inducible promoter" is meant promoter element which is responsible for expression of genes in response to a specific signal, such as: physical stimulus (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites; and stress.

By a "plant" is meant a photosynthetic organism, either eukaryotic and prokaryotic.

By "angiosperm" is meant a plant having its seed enclosed in an ovary (e.g., coffee, tobacco, bean, pea).

By "gymnosperm" is meant a plant having its seed exposed and not enclosed in an ovary (e.g., pine, spruce).

By "monocotyledon" is meant a plant characterized by the presence of only one seed leaf (primary leaf of the embryo). For example, maize, wheat, rice and others.

By "dicotyledon" is meant a plant producing seeds with two cotyledons (primary leaf of the embryo). For example, coffee, canola, peas and others.

By "transgenic plant" is meant a plant expressing a foreign gene.

By "open reading frame" is meant a nucleotide sequence, without introns, encoding an amino acid sequence, with a defined translation initiation and termination region.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high

degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule may be targeted to a highly specific sequence region of a target such that specific gene inhibition can be achieved. Alternatively, enzymatic nucleic acid can be targeted to a highly conserved region of a gene family to inhibit gene expression of a family of related enzymes. The ribozymes can be expressed in plants that have been transformed with vectors which express the nucleic acid of the present invention.

The enzymatic nature of a ribozyme is advantageous over other technologies, since the concentration of ribozyme necessary to affect a therapeutic treatment is lower. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme.

Seven basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first

recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

In one of the preferred embodiments of the inventions herein, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis Δ virus, group I intron, group II intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA. Examples of such hammerhead motifs are described by Dreyfus, supra, Rossi et al., 1992, AIDS Research and Human Retroviruses 8, 183; of hairpin motifs by Hampel et al., EP0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, Feldstein et al., 1989, Gene 82, 53, Haseloff and Gerlach, 1989, Gene, 82, 43, and Hampel et al., 1990 Nucleic Acids Res. 18, 299; of the hepatitis Δ virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNaseP motif by Guerrier-Takada et al., 1983 Cell 35, 849; Forster and Altman, 1990, Science 249, 783; Li and Altman, 1996, Nucleic Acids Res. 24, 835; Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799; Guo and Collins, 1995, EMBO. J. 14, 363); Group II introns are described by Griffin et al., 1995, Chem. Biol. 2, 761; Michels and Pyle, 1995, Biochemistry

34, 2965; and of the Group I intron by Cech et al., U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

The enzymatic nucleic acid molecules of the instant invention will be expressed within cells from eukaryotic promoters [e.g., Gerlach et al., International PCT Publication No. WO 91/13994; Edington and Nelson, 1992, in Gene Regulation: Biology of Antisense RNA and DNA, eds. R. P. Erickson and J. G. Izant, pp 209-221, Raven Press, NY.; Atkins et al., International PCT Publication No. WO 94/00012; Lenee et al., International PCT Publication Nos. WO 94/19476 and WO 9503404, Atkins et al., 1995, J. Gen. Virol. 76, 1781-1790; McElroy and Brettell, 1994, TIBTECH 12, 62; Gruber et al., 1994, J. Cell. Biochem. Suppl. 18A, 110 (X1-406)and Feyter et al., 1996, Mol. Gen. Genet. 250, 329-338; all of these are incorporated by reference herein]. Those skilled in the art will realize from the teachings herein that any ribozyme can be expressed in eukaryotic plant cells from an appropriate promoter. The ribozymes expression is under the control of a constitutive promoter, a tissue-specific promoter or an inducible promoter.

To obtain the ribozyme mediated modulation, the ribozyme RNA is introduced into the plant. There are also numerous ways to transform plants; plants can be transformed using the gene gun (US Patents 4,945,050 to Cornell and 5,141,131 to DowElanco); plants may be

transformed using Agrobacterium technology, see US Patent 5,177,010 to University of Toledo, 5,104,310 to Texas A&M, European Patent Application 0131624B1, European Patent Applications 120516, 159418B1 and 176,112 to Schilperoot, US Patents 5,149,645, 5,469,976, 5,464,763 and 4,940,838 and 4,693,976 to Schilperoot, European Patent Applications 116718, 290799, 320500 all to MaxPlanck, European Patent Applications 604662 and 627752 to Japan Tobacco, European Patent Applications 0267159, and 0292435 and US Patent 5,231,019 all to Ciba Geigy, US Patents 5,463,174 and 4,762,785 both to Calgene, and US Patents 5,004,863 and 5,159,135 both to Agracetus; whiskers technology, see US Patents 5,302,523 and 5,464,765 both to Zeneca; electroporation technology, see WO 87/06614 to Boyce Thompson Institute, 5,472,869 and 5,384,253 both to Dekalb, WO9209696 and WO9321335 both to PGS; all of which are incorporated by reference herein in totality. In addition to numerous technologies for transforming plants, the type of tissue which is contacted with the foreign material (typically plasmids containing RNA or DNA) may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue type I and II, and any tissue which is receptive to transformation and subsequent regeneration into a transgenic plant. Another variable is the choice of a selectable marker. The preference for a particular marker is at the discretion of the artisan, but any of the following selectable markers may be used along with any other gene not listed herein which could function as a selectable marker. Such selectable markers include but are not limited to chlorosulfuron, hygromyacin, PAT and/or bar, bromoxynil, kanamycin and the like. The bar gene may be isolated from Strptomuces, particularly from the hygroscopicus or viridochromogenes

species. The bar gene codes for phosphinothricin acetyl transferase (PAT) that inactivates the active ingradient in the herbicide bialaphos phosphinothricin (PPT). Thus, numerous combinations of technologies may be used in employing ribozyme mediated modulation.

The ribozymes may be expressed individually as monomers, i.e., one ribozyme targeted against one site is expressed per transcript. Alternatively, two or more ribozymes targeted against more than one target site are expressed as part of a single RNA transcript. A single RNA transcript comprising more than one ribozyme targeted against more than one cleavage site are readily generated to achieve efficient modulation of gene expression. Ribozymes within these multimer constructs are the same or different. For example, the multimer construct may comprise a plurality of hammerhead ribozymes or hairpin ribozymes or other ribozyme motifs. Alternatively, the multimer construct may be designed to include a plurality of different ribozyme motifs, such as hammerhead and hairpin ribozymes. More specifically, multimer ribozyme constructs are designed, wherein a series of ribozyme motifs are linked together in tandem in a single RNA transcript. The ribozymes are linked to each other by nucleotide linker sequence, wherein the linker sequence may or may not be complementary to the target RNA. Multimer ribozyme constructs (polyribozymes) are likely to improve the effectiveness of ribozyme-mediated modulation of gene expression.

The activity of ribozymes can also be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595, both hereby incorporated in their totality by reference herein; Ohkawa, J., et al., 1992, Nucleic Acids Symp. Ser., 27, 15-6; Taira, K., et al.,

1991, Nucleic Acids Res., 19, 5125-30; Ventura, M., et al., 1993, Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem. 269, 25856).

Ribozyme-mediated modulation of gene expression can be practiced in a wide variety of plants including but not limited to potato, lettuce spinach, cabbage, brussel sprouts, arugula, kale, collards, chard, beet, turnip, sweet potato and turfgrass. Following are a few non-limiting examples that describe the general utility of ribozymes in modulation of gene expression.

Thus, in one instance, the invention concerns compositions (and methods for their use) for the modulation of genes involved in the biosynthesis of undesirable alkaloid compounds in plants. This is accomplished through the inhibition of genetic expression, with ribozymes, which results in the reduction or elimination of certain gene activities in plants, such as solanidine UDP-glucose glucosyl-transferase. Such activity is reduced in plants, such as potato and other solanaceous plants. These endogenously expressed ribozyme molecules contain substrate binding domains that bind to accessible regions of the target RNA. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, and/or if the level of expression of the target gene is significantly reduced, levels of undesirable alkaloids is reduced or inhibited. Specific examples are provided below in the Tables III and IV.

In one aspect, the ribozymes have binding arms which are complementary to the substrate sequences in Tables

III and IV. Those in the art will recognize that while such examples are designed to one gene RNA (solanidine UDP-glucose glucosyl-transferase) of one plant (e.g., potato), similar ribozymes can be made complementary to other genes in other plant's RNA. By complementary is thus meant that the binding arms of the ribozymes are able to interact with the target RNA in a sequence-specific manner and enable the ribozyme to cause cleavage of a plant mRNA target. Examples of such ribozymes are typically sequences defined in Tables III and IV. The active ribozyme typically contains an enzymatic center equivalent to those in the examples, and binding arms able to bind plant mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such binding and/or cleavage.

In another instance, the invention features compositions (and methods for their use) for the modulation of genes involved in the flower formation in plants. This is accomplished through the inhibition of genetic expression, with ribozymes, which results in the reduction or elimination of certain gene activities in plants, such as citrate synthase. Such activity can be reduced in plants, such as lettuce, spinach, cabbage, brussel sprouts, arugula, kale, collards, chard, beet, turnip, potato, sweet potato and turfgrass. These endogenously expressed ribozyme molecules contain substrate binding domains that bind to accessible regions of the target RNA. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, and/or if the level of expression of the target gene is

significantly reduced, levels of undesirable alkaloids is reduced or inhibited. Specific examples are provided below in the Tables V and VI. In a non-limiting example, ribozymes have binding arms which are complementary to the substrate sequences shown in Tables V and VI are disclosed. Those in the art will recognize that while such examples are designed to one gene RNA (citrate synthase) of one plant (e.g., potato), similar ribozymes can be made complementary to other genes in other plant's RNA. By complementary is thus meant that the binding arms of the ribozymes are able to interact with the target RNA in a sequence-specific manner and enable the ribozyme to cause cleavage of a plant mRNA target. Examples of such ribozymes are typically sequences defined in Tables V and VI. The active ribozyme typically contains an enzymatic center equivalent to those in the examples, and binding arms able to bind plant mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such binding and/or cleavage.

The sequences of the ribozymes that are particularly useful in this study, are shown in Tables III-VI.

Those in the art will recognize that ribozyme sequences listed in the Tables are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Table III and V (5'-GGCGAAAGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences, preferably provided that a minimum of a two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Table IV and VI (5'-CACGUUGUG-3') can be altered (substitution, deletion,

and/or insertion) to contain any sequence, preferably provided that a minimum of a two base-paired stem structure can form. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

Preferably, the recombinant vectors capable of stable integration into the plant genome and selection of transformed plant lines expressing the ribozymes are expressed either by constitutive or inducible promoters in the plant cells. Once expressed, the ribozymes cleave their target mRNAs and reduce alkaloid production in their host cells. The ribozymes expressed in plant cells are under the control of a constitutive promoter, a tissue-specific promoter or an inducible promoter.

Modification of undesirable alkaloid profile is an important application of nucleic acid-based technologies which are capable of reducing specific gene expression. A high level of undesirable alkaloid compounds is undesirable in plants that produce products of commercial importance.

In preferred embodiments, hairpin and hammerhead ribozymes that cleave solanidine UDP-glucose glucosyl-transferase RNA are described. Those of ordinary skill in the art will understand from the examples described below that other ribozymes that cleave target RNAs required for solanidine UDP-glucose glucosyl-transferase activity may now be readily designed and are within the scope of the invention.

Modification of flower formation is an important application of nucleic acid-based technologies which are capable of reducing specific gene expression. In preferred embodiments, hairpin and hammerhead ribozymes that cleave potato citrate synthase RNA are described. Those of ordinary skill in the art will understand from the examples described below that other ribozymes that

cleave target RNAs required for potato citrate synthase activity may now be readily designed and are within the scope of the invention

While specific examples to potato RNA are provided, those in the art will recognize that the teachings are not limited to potato. Furthermore, the same or equivalent target may be used in other plant species. The complementary arms suitable for targeting the specific plant RNA sequences are utilized in the ribozyme targeted to that specific RNA. The examples and teachings herein are meant to be non-limiting, and those skilled in the art will recognize that similar embodiments can be readily generated in a variety of different plants to modulate expression of a variety of different genes, using the teachings herein, and are within the scope of the inventions.

Standard molecular biology techniques were followed in the examples herein. Additional information may be found in Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989), Molecular Cloning a Laboratory Manual, second edition, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, which is incorporated herein by reference.

Examples

Example 1: Identification of Potential Ribozyme Cleavage Sites for solanidine UDP-glucose glucosyl-transferase

Approximately 353 HH ribozyme cleavage sites and approximately 20 HP sites were identified in the potato solanidine UDP-glucose glucosyl-transferase RNA. A HH site consists of a uridine and any nucleotide except guanosine (UH). Tables III and IV have a list of HH and HP ribozyme cleavage sites. The numbering system starts with 1 at the 5' end of a solanidine UDP-glucose

glucosyl-transferase RNA having the sequence shown in Moehs et al., supra.

Ribozymes, such as those listed in Tables III and IV, can be readily designed and synthesized to such cleavage sites with between 5 and 100 or more bases as substrate binding arms (see Figs. 1 - 5). These substrate binding arms within a ribozyme allow the ribozyme to interact with their target in a sequence-specific manner.

Example 2: Selection of Ribozyme Cleavage Sites for solanidine UDP-glucose glucosyl-transferase

The secondary structure of solanidine UDP-glucose glucosyl-transferase RNA was assessed by computer analysis using algorithms, such as those developed by M. Zuker (Zuker, M., 1989 Science, 244, 48-52). Regions of the mRNA that did not form secondary folding structures with RNA/RNA stems of over eight nucleotides and contained potential hammerhead ribozyme cleavage sites were identified.

Example 3: Hammerhead and Hairpin Ribozymes for solanidine UDP-glucose glucosyl-transferase

Hammerhead (HH) and hairpin (HP) ribozymes are subjected to analysis by computer folding and the ribozymes that had significant secondary structure are rejected.

The ribozymes are chemically synthesized. The general procedures for RNA synthesis have been described previously (Usman et al., 1987, J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990, Nucl. Acids Res., 18, 5433-5341; Wincott et al., 1995, Nucleic Acids Res. 23, 2677). Small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a modified 2.5

μmol scale protocol with a 5 min coupling step for alkylsilyl protected nucleotides and 2.5 min coupling step for 2'-O-methylated nucleotides. Table II outlines the amounts, and the contact times, of the reagents used in the synthesis cycle. A 6.5-fold excess (163 μL of 0.1 M = 16.3 μmol) of phosphoramidite and a 24-fold excess of S-ethyl tetrazole (238 μL of 0.25 M = 59.5 μmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride (ABI); capping was performed with 16% N-Methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (Millipore). B & J Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from American International Chemical, Inc.

Deprotection of the RNA is performed as follows. The polymer-bound oligoribonucleotide, trityl-off, is transferred from the synthesis column to a 4 mL glass screw top vial and suspended in a solution of methylamine (MA) at 65°C for 10 min. After cooling to -20°C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H_2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The base-deprotected oligoribonucleotide is resuspended in anhydrous TEA•HF/NMP solution (250 μL of a

solution of 1.5 mL N-methylpyrrolidinone, 750 µL TEA and 1.0 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65°C for 1.5 h. The resulting, fully deprotected, oligomer is quenched with 50 mM TEAB (9 mL) prior to anion exchange desalting.

For anion exchange desalting of the deprotected oligomer, the TEAB solution is loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) that is prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA is eluted with 2 M TEAB (10 mL) and dried down to a white powder.

Inactive hammerhead ribozymes are synthesized by substituting a U for G_5 and a U for A_{14} (numbering from (Hertel, K. J., et al., 1992, Nucleic Acids Res., 20, 3252).

The hairpin ribozymes are synthesized as described above for the hammerhead RNAs.

Ribozymes can also synthesized be from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180, 51). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Wincott et al., 1996, supra, the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in Tables III and IV.

Example 4: Construction of Ribozyme expressing transcription units for solanidine UDP-glucose glucosyl-transferase

Ribozymes targeted to cleave solanidine UDP-glucose glucosyl-transferase RNA can be endogenously expressed in

plants, either from genes inserted into the plant genome (stable transformation) or from episomal transcription units (transient expression) which are part of plasmid vectors or viral sequences. These ribozymes can be expressed via RNA polymerase I, II, or III plant or plant virus promoters (such as CaMV). Promoters can be either constitutive, tissue specific, or developmentally expressed.

Example 5: Identification of Potential Ribozyme Cleavage Sites for potato citrate synthase

Approximately 398 HH ribozyme cleavage sites and approximately 25 HP sites were identified in the potato citrate synthase RNA. A HH site consists of a uridine and any nucleotide except guanosine (UH). Tables V and VI have a list of HH and HP ribozyme cleavage sites.

Ribozymes, such as those listed in Tables III and IV, can be readily designed and synthesized to such cleavage sites with between 5 and 100 or more bases as substrate binding arms (see Figs. 1 - 5). These substrate binding arms within a ribozyme allow the ribozyme to interact with their target in a sequence-specific manner.

Example 6: Selection of Ribozyme Cleavage Sites for potato citrate synthase

The secondary structure of potato citrate synthase RNA was assessed by computer analysis using algorithms, such as those developed by M. Zuker (Zuker, M., 1989 Science, 244, 48-52). Regions of the mRNA that did not form secondary folding structures with RNA/RNA stems of over eight nucleotides and contained potential hammerhead ribozyme cleavage sites were identified.

Example 7: Hammerhead and Hairpin Ribozymes for potato citrate synthase

Hammerhead (HH) and hairpin (HP) ribozymes are subjected to analysis by computer folding and the ribozymes that had significant secondary structure are rejected.

The ribozymes are synthesized as described above. The sequences of the chemically synthesized ribozymes used in this study are shown below in Tables V and VI.

Example 8: Construction of Ribozyme expressing transcription units for potato citrate synthase

Ribozymes targeted to cleave potato citrate synthase RNA can be endogenously expressed in plants, either from genes inserted into the plant genome (stable transformation) or from episomal transcription units (transient expression) which are part of plasmid vectors or viral sequences. These ribozymes can be expressed via RNA polymerase I, II, or III plant or plant virus promoters (such as CaMV). Promoters can be either constitutive, tissue specific, or developmentally expressed.

Example 9: Plant Transformation and Construction

There are several methods to genetically engineer plants (for a review see Gasser et al., 1989 Science 244, 1293-1299; Potrykus, 1991 Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 205-225; Gasser and Fraley, 1992 Scientific American June 1992 pp 62-69). These methods can be used to introduce the above ribozymes directly or via exression vectors. These methods include the following:

Helium blasting involves accelerating suspended DNA-coated gold particles towards and into prepared tissue targets. The device used was an earlier prototype to the one described in a DowElanco U.S. Patent (#5,141,131) which is incorporated herein by reference, although both function in a similar manner. The device consists of a high pressure helium source, a syringe containing the DNA/gold suspension, and a pneumatically-operated multipurpose valve which provides controlled linkage between the helium source and a loop of pre-loaded DNA/gold suspension. Prior to blasting, tissue targets are covered with a sterile 104 micron stainless steel screen, which holds the tissue in place during impact. Next, targets are placed under vacuum in the main chamber of the device. The DNA-coated gold particles are accelerated at the target 4 times using a helium pressure of 1500 psi. Each blast delivered 20 µl of DNA/gold suspension. Immediately post-blasting, the targets are placed back on maintenance medium plus osmoticum for a 16 to 24 hour recovery period.

Particle Bombardment-mediated transformation (Gordon-Kamm et al., 1990 The Plant Cell 2, 603-618; Potrykus, 1991 Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 205-225; Gasser and Fraley, 1992 Scientific American June 1992 pp 62-69; Vain et al., 1993 Plant Cell Rep. 12, 84-88; Weymann et al., 1993 In Vitro Cell. Dev. Biol. 29P, 33-37): This strategy involves bombardment of plant cells with minute (1-2 microns in diameter) metal particles (for example tungsten or gold particles) using a "gene" gun (also referred to as "Biolistics" or "particle" gun). The metal particles, coated with genetic material (ribozyme or ribozyme encoding plasmids), can penetrate the cell wall, without causing

any irreversible damage to the cell, and deliver the genetic material to the cytoplasm.

Electroporation-mediated transformation (Fromm et al., 1986 Nature 319, 791-793; Rhodes et al., 1988 Science 240, 204-207; Potrykus, 1991 Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 205-225; Gasser and Fraley, 1992 Scientific American June 1992 pp 62-69; D'Halluin et al., 1992 The Plant Cell 4, 1495-1505; Sukhapinda et al., 1993 Plant Cell Rep. 13, 63-68; Laursen et al., 1994 Plant Mol. Biol. 24, 51-61): This technique involves permeabilizing the target cell membrane by using short high voltage electric pulses. Nucleic acids (ribozyme encoding plasmids) can pass through a permeabilized cell membrane and potentially integrate into the host genome resulting in a transformed phenotype. Electroporation can be carried out on (a) plant protoplasts, plant cells lacking a cell wall, (Fromm et al., 1986 Nature 319, 791-793; Rhodes et al., 1988 Science 240, 204-207; Sukhapinda et al., 1993 Plant Cell Rep. 13, 63-68); (b) cultured cells (Laursen et al., 1994 Plant Mol. Biol. 24, 51-61); (c) Plant tissue (D'Halluin et al., 1992 The Plant Cell 4, 1495-1505).

Agrobacterium-mediated transformation: This method uses a disarmed (disease causing genes are deleted) species of Agrobacterium tumefaciens or Agrobacterium rizogenes (Potrykus, 1991 Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 205-225; Gasser and Fraley, 1992 Scientific American June 1992 pp 62-69). This organism transfers part of its DNA into plant cells (T-DNA). Ribozyme genes can be cloned into T-DNA fragments and Agrobacterium containing the recombinant T-DNA can be generated. Agrobacterium will infect and release the recombinant T- DNA into maize cells. The integration of

T-DNA into host DNA will result in a transformed phenotype.

Other Uses:

Potential usefulness of sequence-specific enzymatic nucleic acid molecules of the instant invention might have many of the same applications for the study of RNA that DNA restriction endonucleases have for the study of DNA (Nathans, D. and Smith, H. O., (1975) Ann. Rev. Biochem. 44:273). For example, the pattern of restriction fragments could be used to establish sequence relationships between two related plant RNAs, and large plant RNAs could be specifically cleaved to fragments of a size more useful for study. The ability to engineer sequence specificity of the ribozyme is ideal for cleavage of RNAs of unknown sequence.

Ribozymes of this invention may be used as tools to examine genetic drift and mutations within plant cells. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the synthesis of undesirable alkaloids in plants. In this manner, other genetic targets may be defined as important mediators of alkaloid production. These experiments will lead to better modifications of the alkaloid production by affording the possibility of combinational concepts (e.g., multiple ribozymes targeted

to different genes intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNA associated with undesirable alkaloid production condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

Other embodiments are within the following claims.

Table I

Table I:
Characteristics of naturally occurring ribozymes

Group I Introns

- Size: ~150 to >1000 nucleotides.
- Requires a U in the target sequence immediately 5' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site.
- Reaction mechanism: attack by the 3'-011 of guanosine to generate cleavage products with 3'-OH and 5'-guanosine.
- Additional protein cofactors required in some cases to help folding and maintainance of the active structure [1].
- Over 300 known members of this class. Found as an intervening sequence in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.
- Major structural features largely established through phylogenetic comparisons, mutagenesis, and biochemical studies [2,3].
- Complete kinetic framework established for one ribozyme [4,5,6,7]
- Studies of ribozyme folding and substrate docking underway [8,9,10].
- Chemical modification investigation of important residues well established [11,12].
- The small (4-6 nt) binding site may make this ribozyme too non-specific for targeted RNA cleavage, however, the Tetrahymena group I intron has been used to repair a "defective" ß-galactosidase message by the ligation

Table I

of new ß-galactosidase sequences onto the defective message [13].

RNAse P RNA (M1 RNA)

- Size: ~290 to 400 nucleotides.
- RNA portion of a ubiquitous ribonucleoprotein enzyme.
- Cleaves tRNA precursors to form mature tRNA [14].
- Reaction mechanism: possible attack by M^{2+}-OH to generate cleavage products with 3'OH and 5'-phosphate.
- RNAse P is found throughout the prokaryotes and eukaryotes. The RNA subunit has been sequenced from bacteria, yeast, rodents, and primates.
- Recruitment of endogenous RNAse P for therapeutic applications is possible through hybridization of an External Guide Sequence (EGS) to the target RNA
- Important phosphate and 2' OH contacts recently identified [17,18]

Group II Introns

- Size: >1000 nucleotides.
- Trans cleavage of target RNAs recently demonstrated [19,20].
- Sequence requirements not fully determined.
- Reaction mechanism: 2'-OH of an internal adenosine generates cleavage products with 3'-OH and a "lariat" RNA containing a 3'-5' and a 2'-5' branch point.
- Only natural ribozyme with demonstrated participation in DNA cleavage [21,22] in addition to RNA cleavage and ligation.
- Major structural features largely established through phylogenetic comparisons [23].

Table I

- Important 2' OH contacts beginning to be identified [24]
- Kinetic framework under development [25]

Neurospora VS RNA

- Size: ~144 nucleotides.
- Trans cleavage of hairpin target RNAs recently demonstrated [26]
- Sequence requirements not fully determined.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Binding sites and structural requirements not fully determined.
- Only 1 known member of this class. Found in Neurospora VS RNA.

Hammerhead Ribozyme

(see text for references)

- Size: ~13 to 40 nucleotides.
- Requires the target sequence UH immediately 5' of the cleavage site.
- Binds a variable number nucleotides on both sides of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent.
- Essential structural features largely defined, including 2 crystal structures []

Table I

- Minimal ligation activity demonstrated (for engineering through in vitro selection) []
- Complete kinetic framework established for two or more ribozymes [].
- Chemical modification investigation of important residues well established [].

Hairpin Ribozyme

- Size: ~50 nucleotides.
- Requires the target sequence GUC immediately 3′ of the cleavage site.
- Binds 4-6 nucleotides at the 5′-side of the cleavage site and a variable number to the 3′- side of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 3 known members of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent.
- Essential structural features largely defined [27,28,29,30]
- Ligation activity (in addition to cleavage activity) makes ribozyme amenable to engineering through in vitro selection [31]
- Complete kinetic framework established for one ribozyme [31].
- Chemical modification investigation of important residues begun [33,34]

Table I

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [31].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [36].
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2,3'-cyclic phosphate and 5'-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [37]

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Table II: 2.5 µmol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 µL	2.5
S-Ethyl Tetrazole	23.8	238 µL	2.5
Acetic Anhydride	100	233 µL	5 sec
N-Methyl Imidazole	186	233 µL	5 sec
TCA	83.2	1.73 mL	21 sec
Iodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

* Wait time does not include contact time during delivery.

Table III

Table III: Solanidine glucosyltransferase Hammerhead Ribozyme and Target Sequences

Nt. Position	Substrate	Ribozyme
13	UCUUGGGUA GUAAAAAU	AUUUUUAC CUGAUGA X GAA ACCCAAGA
16	UGGGUAGUA AAAAUGGU	ACCAUUUU CUGAUGA X GAA ACUACCCA
25	AAAAUGGUA GCAACCUG	CAGGUUGC CUGAUGA X GAA ACCAUUUU
49	GGCGAAAUC CUCCAUGU	ACAUGGAG CUGAUGA X GAA AUUUCGCC
52	GAAAUCCUC CAUGUUCU	AGAACAUG CUGAUGA X GAA AGGAUUUC
58	CUCCAUGUU CUUUUCCU	AGGAAAAG CUGAUGA X GAA ACAUGGAG
59	UCCAUGUUC UUUUCCUU	AAGGAAAA CUGAUGA X GAA AACAUGGA
61	CAUGUUCUU UUCCUUCC	GGAAGGAA CUGAUGA X GAA AGAACAUG
62	AUGUUCUUU UCCUUCCC	GGGAAGGA CUGAUGA X GAA AAGAACAU
63	UGUUCUUUU CCUUCCCU	AGGGAAGG CUGAUGA X GAA AAAGAACA
64	GUUCUUUUC CUUCCCUU	AAGGGAAG CUGAUGA X GAA AAAAGAAC
67	CUUUUCCUU CCCUUCUU	AAGAAGGG CUGAUGA X GAA AGGAAAAG
68	UUUUCCUUC CCUUCUUA	UAAGAAGG CUGAUGA X GAA AAGGAAAA
72	CCUUCCCUU CUUAUCCG	CGGAUAAG CUGAUGA X GAA AGGGAAGG
73	CUUCCCUUC UUAUCCGC	GCGGAUAA CUGAUGA X GAA AAGGGAAG
75	UCCCUUCUU AUCCGCUG	CAGCGGAU CUGAUGA X GAA AGAAGGGA
76	CCCUUCUUA UCCGCUGG	CCAGCGGA CUGAUGA X GAA AAGAAGGG
78	CUUCUUAUC CGCUGGUC	GACCAGCG CUGAUGA X GAA AUAAGAAG
86	CCGCUGGUC AUUUCAUC	GAUGAAAU CUGAUGA X GAA ACCAGCGG
89	CUGGUCAUU UCAUCCCA	UGGGAUGA CUGAUGA X GAA AUGACCAG
90	UGGUCAUUU CAUCCCAU	AUGGGAUG CUGAUGA X GAA AAUGACCA
91	GGUCAUUUC AUCCCAUU	AAUGGGAU CUGAUGA X GAA AAAUGACC
94	CAUUUCAUC CCAUUAGU	ACUAAUGG CUGAUGA X GAA AUGAAAUG
99	CAUCCCAUU AGUUAACG	CGUUAACU CUGAUGA X GAA AUGGGAUG
100	AUCCCAUUA GUUAACGC	GCGUUAAC CUGAUGA X GAA AAUGGGAU
103	CCAUUAGUU AACGCCGC	GCGGCGUU CUGAUGA X GAA ACUAAUGG
104	CAUUAGUUA ACGCCGCA	UGCGGCGU CUGAUGA X GAA AACUAAUG
118	GCAAGGCUA UUCGCCUC	GAGGCGAA CUGAUGA X GAA AGCCUUGC
120	AAGGCUAUU CGCCUCCC	GGGAGGCG CUGAUGA X GAA AUAGCCUU
121	AGGCUAUUC GCCUCCCG	CGGGAGGC CUGAUGA X GAA AAUAGCCU
126	AUUCGCCUC CCGGGUGU	ACACCCGG CUGAUGA X GAA AGGCGAAU
135	CCGGGUGUU AAAGCCAC	GUGGCUUU CUGAUGA X GAA ACACCCGG
136	CGGGUGUUA AAGCCACA	UGUGGCUU CUGAUGA X GAA AACACCCG
147	GCCACAAUC CUCACUAC	GUAGUGAG CUGAUGA X GAA AUUGUGGC
150	ACAAUCCUC ACUACCCC	GGGGUAGU CUGAUGA X GAA AGGAUUGU
154	UCCUCACUA CCCCUCAU	AUGAGGGG CUGAUGA X GAA AGUGAGGA
160	CUACCCCUC AUAAUGCC	GGCAUUAU CUGAUGA X GAA AGGGGUAG
163	CCCCUCAUA AUGCCUUA	UAAGGCAU CUGAUGA X GAA AUGAGGGG
170	UAAUGCCUU ACUUUUUA	UAAAAAGU CUGAUGA X GAA AGGCAUUA
171	AAUGCCUUA CUUUUUAG	CUAAAAAG CUGAUGA X GAA AAGGCAUU
174	GCCUUACUU UUUAGAUC	GAUCUAAA CUGAUGA X GAA AGUAAGGC
175	CCUUACUUU UUAGAUCU	AGAUCUAA CUGAUGA X GAA AAGUAAGG
176	CUUACUUUU UAGAUCUA	UAGAUCUA CUGAUGA X GAA AAAGUAAG
177	UUACUUUUU AGAUCUAC	GUAGAUCU CUGAUGA X GAA AAAAGUAA
178	UACUUUUUA GAUCUACU	AGUAGAUC CUGAUGA X GAA AAAAAGUA
182	UUUUAGAUC UACUAUUG	CAAUAGUA CUGAUGA X GAA AUCUAAAA
184	UUAGAUCUA CUAUUGAC	GUCAAUAG CUGAUGA X GAA AGAUCUAA
187	GAUCUACUA UUGACGAU	AUCGUCAA CUGAUGA X GAA KGUAGAUC
189	UCUACUAUU GACGAUGA	UCAUCGUC CUGAUGA X GAA AUAGUAGA
201	GAUGAUGUU CGAAUUUC	GAAAUUCG CUGAUGA X GAA ACAUCAUC

Table III

Nt. Position	Substrate	Ribozyme
202	AUGAUGUUC GAAUUUCC	GGAAAUUC CUGAUGA X GAA AACAUCAU
207	GUUCGAAUU UCCGGAUU	AAUCCGGA CUGAUGA X GAA AUUCGAAC
208	UUCGAAUUU CCGGAUUU	AAAUCCGG CUGAUGA X GAA AAUUCGAA
209	UCGAAUUUC CGGAUUUC	GAAAUCCG CUGAUGA X GAA AAAUUCGA
215	UUCCGGAUU UCCCAUUU	AAAUGGGA CUGAUGA X GAA AUCCGGAA
216	UCCGGAUUU CCCAUUUC	GAAAUGGG CUGAUGA X GAA AAUCCGGA
217	CCGGAUUUC CCAUUUCU	AGAAAUGG CUGAUGA X GAA AAAUCCGG
222	UUUCCCAUU UCUAUCGU	ACGAUAGA CUGAUGA X GAA AUGGGAAA
223	UUCCCAUUU CUAUCGUA	UACGAUAG CUGAUGA X GAA AAUGGGAA
224	UCCCAUUUC UAUCGUAA	UUACGAUA CUGAUGA X GAA AAAUGGGA
226	CCAUUUCUA UCGUAACU	AGUUACGA CUGAUGA X GAA AGAAAUGG
228	AUUUCUAUC GUAACUAU	AUAGUUAC CUGAUGA X GAA AUAGAAAU
231	UCUAUCGUA ACUAUUAA	UUAAUAGU CUGAUGA X GAA ACGAUAGA
235	UCGUAACUA UUAAAUUC	GAAUUUAA CUGAUGA X GAA AGUUACGA
237	GUAACUAUU AAAUUCCC	GGGAAUUU CUGAUGA X GAA AUAGUUAC
238	UAACUAUUA AAUUCCCC	GGGGAAUU CUGAUGA X GAA AAUAGUUA
242	UAUUAAAUU CCCCUCUG	CAGAGGGG CUGAUGA X GAA AUUUAAUA
243	AUUAAAUUC CCCUCUGC	GCAGAGGG CUGAUGA X GAA AAUUUAAU
248	AUUCCCCUC UGCUGAAG	CUUCAGCA CUGAUGA X GAA AGGGGAAU
258	GCUGAAGUU GGGUUGCC	GGCAACCC CUGAUGA X GAA ACUUCAGC
263	AGUUGGGUU GCCUGAAG	CUUCAGGC CUGAUGA X GAA ACCCAACU
276	GAAGGAAUU GAGAGCUU	AAGCUCUC CUGAUGA X GAA AUUCCUUC
284	UGAGAGCUU UAACUCUG	CAGAGUUA CUGAUGA X GAA AGCUCUCA
285	GAGAGCUUU AACUCUGC	GCAGAGUU CUGAUGA X GAA AAGCUCUC
286	AGAGCUUUA ACUCUGCC	GGCAGAGU CUGAUGA X GAA AAAGCUCU
290	CUUUAACUC UGCCACUU	AAGUGGCA CUGAUGA X GAA AGUUAAAG
298	CUGCCACUU CACCUGAA	UUCAGGUG CUGAUGA X GAA AGUGGCAG
299	UGCCACUUC ACCUGAAA	UUUCAGGU CUGAUGA X GAA AAGUGGCA
313	AAAUGCCUC AUAAAAUU	AAUUUUAU CUGAUGA X GAA AGGCAUUU
316	UGCCUCAUA AAAUUUUU	AAAAAUUU CUGAUGA X GAA AUGAGGCA
321	CAUAAAAUU UUUUAUGC	GCAUAAAA CUGAUGA X GAA AUUUUAUG
322	AUAAAAUUU UUUAUGCU	AGCAUAAA CUGAUGA X GAA AAUUUUAU
323	UAAAAUUUU UUAUGCUC	GAGCAUAA CUGAUGA X GAA AAAUUUUA
324	AAAAUUUUU UAUGCUCU	AGAGCAUA CUGAUGA X GAA AAAAUUUU
325	AAAUUUUUU AUGCUCUU	AAGAGCAU CUGAUGA X GAA AAAAAUUU
326	AAUUUUUUA UGCUCUUU	AAAGAGCA CUGAUGA X GAA AAAAAAUU
331	UUUAUGCUC UUUCUCUU	AAGAGAAA CUGAUGA X GAA AGCAUAAA
333	UAUGCUCUU UCUCUUCU	AGAAGAGA CUGAUGA X GAA AGAGCAUA
334	AUGCUCUUU CUCUUCUA	UAGAAGAG CUGAUGA X GAA AAGAGCAU
335	UGCUCUUUC UCUUCUAC	GUAGAAGA CUGAUGA X GAA AAAGAGCA
337	CUCUUUCUC UUCUACAA	UUGUAGAA CUGAUGA X GAA AGAAAGAG
339	CUUUCUCUU CUACAAAA	UUUUGUAG CUGAUGA X GAA AGAGAAAG
340	UUUCUCUUC UACAAAAG	CUUUUGUA CUGAUGA X GAA AAGAGAAA
342	UCUCUUCUA CAAAAGCC	GGCUUUUG CUGAUGA X GAA AGAAGAGA
361	UGGAAGAUA AAAUUCGU	ACGAAUUU CUGAUGA X GAA AUCUUCCA
366	GAUAAAAUU CGUGAACU	AGUUCACG CUGAUGA X GAA AUUUUAUC
367	AUAAAAUUC GUGAACUC	GAGUUCAC CUGAUGA X GAA AAUUUUAU
375	CGUGAACUC CGUCCUGA	UCAGGACG CUGAUGA X GAA AGUUCACG
379	AACUCCGUC CUGAUUGC	GCAAUCAG CUGAUGA X GAA ACGGAGUU
385	GUCCUGAUU GCAUUUUU	AAAAAUGC CUGAUGA X GAA AUCAGGAC
390	GAUUGCAUU UUUUCUGA	UCAGAAAA CUGAUGA X GAA AUGCAAUC
391	AUUGCAUUU UUUCUGAU	AUCAGAAA CUGAUGA X GAA AAUGCAAU
392	UUGCAUUUU UUCUGAUA	UAUCAGAA CUGAUGA X GAA AAAUGCAA
393	UGCAUUUUU UCUGAUAU	AUAUCAGA CUGAUGA X GAA AAAAUGCA
394	GCAUUUUUU CUGAUAUG	CAUAUCAG CUGAUGA X GAA AAAAAUGC
395	CAUUUUUUC UGAUAUGU	ACAUAUCA CUGAUGA X GAA AAAAAAUG
400	UUUCUGAUA UGUACUUC	GAAGUACA CUGAUGA X GAA AUCAGAAA

Table III

Nt. Position	Substrate	Ribozyme
404	UGAUAUGUA CUUCCCUU	AAGGGAAG CUGAUGA X GAA ACAUAUCA
407	UAUGUACUU CCCUUGGA	UCCAAGGG CUGAUGA X GAA AGUACAUA
408	AUGUACUUC CCUUGGAC	GUCCAAGG CUGAUGA X GAA AAGUACAU
412	ACUUCCCUU GGACAGUA	UACUGUCC CUGAUGA X GAA AGGGAAGU
420	UGGACAGUA GAUAUUGC	GCAAUAUC CUGAUGA X GAA ACUGUCCA
424	CAGUAGAUA UUGCUGAU	AUCAGCAA CUGAUGA X GAA AUCUACUG
426	GUAGAUAUU GCUGAUGA	UCAUCAGC CUGAUGA X GAA AUAUCUAC
438	GAUGAGCUU CACAUCCC	GGGAUGUG CUGAUGA X GAA AGCUCAUC
439	AUGAGCUUC ACAUCCCU	AGGGAUGU CUGAUGA X GAA AAGCUCAU
444	CUUCACAUC CCUCGUAU	AUACGAGG CUGAUGA X GAA AUGUGAAG
448	ACAUCCCUC GUAUUUUG	CAAAAUAC CUGAUGA X GAA AGGGAUGU
451	UCCCUCGUA UUUUGUAC	GUACAAAA CUGAUGA X GAA ACGAGGGA
453	CCUCGUAUU UUGUACAA	UUGUACAA CUGAUGA X GAA AUACGAGG
454	CUCGUAUUU UGUACAAU	AUUGUACA CUGAUGA X GAA AAUACGAG
455	UCGUAUUUU GUACAAUU	AAUUGUAC CUGAUGA X GAA AAAUACGA
458	UAUUUUGUA CAAUUUGU	ACAAAUUG CUGAUGA X GAA ACAAAAUA
463	UGUACAAUU UGUCUGCU	AGCAGACA CUGAUGA X GAA AUUGUACA
464	GUACAAUUU GUCUGCUU	AAGCAGAC CUGAUGA X GAA AAUUGUAC
467	CAAUUUGUC UGCUUACA	UGUAAGCA CUGAUGA X GAA ACAAAUUG
472	UGUCUGCUU ACAUGUGC	GCACAUGU CUGAUGA X GAA AGCAGACA
473	GUCUGCUUA CAUGUGCU	AGCACAUG CUGAUGA X GAA AAGCAGAC
482	CAUGUGCUA CAGCAUUA	UAAUGCUG CUGAUGA X GAA AGCACAUG
489	UACAGCAUU AUGCACAA	UUGUGCAU CUGAUGA X GAA AUGCUGUA
490	ACAGCAUUA UGCACAAC	GUUGUGCA CUGAUGA X GAA AAUGCUGU
501	CACAACCUU AAGGUUUA	UAAACCUU CUGAUGA X GAA AGGUUGUG
502	ACAACCUUA AGGUUUAC	GUAAACCU CUGAUGA X GAA AAGGUUGU
507	CUUAAGGUU UACAGACC	GGUCUGUA CUGAUGA X GAA ACCUUAAG
508	UUAAGGUUU ACAGACCU	AGGUCUGU CUGAUGA X GAA AACCUUAA
509	UAAGGUUUA CAGACCUC	GAGGUCUG CUGAUGA X GAA AAACCUUA
517	ACAGACCUC ACAAGCAG	CUGCUUGU CUGAUGA X GAA AGGUCUGU
529	AGCAGCCUA AUCUAGAC	GUCUAGAU CUGAUGA X GAA AGGCUGCU
532	AGCCUAAUC UAGACGAA	UUCGUCUA CUGAUGA X GAA AUUAGGCU
534	CCUAAUCUA GACGAAUC	GAUUCGUC CUGAUGA X GAA AGAUUAGG
542	AGACGAAUC UCAAAGUU	AACUUUGA CUGAUGA X GAA AUUCGUCU
544	ACGAAUCUC AAAGUUUC	GAAACUUU CUGAUGA X GAA AGAUUCGU
550	CUCAAAGUU UCGUGGUU	AACCACGA CUGAUGA X GAA ACUUUGAG
551	UCAAAGUUU CGUGGUUC	GAACCACG CUGAUGA X GAA AACUUUGA
552	CAAAGUUUC GUGGUUCC	GGAACCAC CUGAUGA X GAA AAACUUUG
558	UUCGUGGUU CCUGGUUU	AAACCAGG CUGAUGA X GAA ACCACGAA
559	UCGUGGUUC CUGGUUUA	UAAACCAG CUGAUGA X GAA AACCACGA
565	UUCCUGGUU UACCUGAU	AUCAGGUA CUGAUGA X GAA ACCAGGAA
566	UCCUGGUUU ACCUGAUG	CAUCAGGU CUGAUGA X GAA AACCAGGA
567	CCUGGUUUA CCUGAUGA	UCAUCAGG CUGAUGA X GAA AAACCAGG
579	GAUGAGAUA AAGUUCAA	UUGAACUU CUGAUGA X GAA AUCUCAUC
584	GAUAAAGUU CAAGUUAU	AUAACUUG CUGAUGA X GAA ACUUUAUC
585	AUAAAGUUC AAGUUAUC	GAUAACUU CUGAUGA X GAA AACUUUAU
590	GUUCAAGUU AUCCCAAC	GUUGGGAU CUGAUGA X GAA ACUUGAAC
591	UUCAAGUUA UCCCAACU	AGUUGGGA CUGAUGA X GAA AACUUGAA
593	CAAGUUAUC CCAACUGA	UCAGUUGG CUGAUGA X GAA AUAACUUG
610	CAGAUGAUC UGAGAAAG	CUUUCUCA CUGAUGA X GAA AUCAUCUG
620	GAGAAAGUC GGAUGACC	GGUCAUCC CUGAUGA X GAA ACUUUCUC
639	AAGACUGUU UUUGACGA	UCGUCAAA CUGAUGA X GAA ACAGUCUU
640	AGACUGUUU UUGACGAA	UUCGUCAA CUGAUGA X GAA AACAGUCU
641	GACUGUUUU UGACGAAU	AUUCGUCA CUGAUGA X GAA AAACAGUC
642	ACUGUUUUU GACGAAUU	AAUUCGUC CUGAUGA X GAA AAAACAGU
650	UGACGAAUU GCUCGAAC	GUUCGAGC CUGAUGA X GAA AUUCGUCA
654	GAAUUGCUC GAACAAGU	ACUUGUUC CUGAUGA X GAA AGCAAUUC

Table III

Nt. Position	Substrate	Ribozyme
663	GAACAAGUU GAAGAUUC	GAAUCUUC CUGAUGA X GAA ACUUGUUC
670	UUGAAGAUU CGGAGGAA	UUCCUCCG CUGAUGA X GAA AUCUUCAA
671	UGAAGAUUC GGAGGAAC	GUUCCUCC CUGAUGA X GAA AAUCUUCA
686	ACGAAGCUA UGGCAUUG	CAAUGCCA CUGAUGA X GAA AGCUUCGU
693	UAUGGCAUU GUUCAUGA	UCAUGAAC CUGAUGA X GAA AUGCCAUA
696	GGCAUUGUU CAUGAUAC	GUAUCAUG CUGAUGA X GAA ACAAUGCC
697	GCAUUGUUC AUGAUACA	UGUAUCAU CUGAUGA X GAA AACAAUGC
703	UUCAUGAUA CAUUUUAU	AUAAAAUG CUGAUGA X GAA AUCAUGAA
707	UGAUACAUU UUAUGAGC	GCUCAUAA CUGAUGA X GAA AUGUAUCA
708	GAUACAUUU UAUGAGCU	AGCUCAUA CUGAUGA X GAA AAUGUAUC
709	AUACAUUUU AUGAGCUA	UAGCUCAU CUGAUGA X GAA AAAUGUAU
710	UACAUUUUA UGAGCUAG	CUAGCUCA CUGAUGA X GAA AAAAUGUA
717	UAUGAGCUA GAACCUGC	GCAGGUUC CUGAUGA X GAA AGCUCAUA
728	ACCUGCAUA UGUUGACU	AGUCAACA CUGAUGA X GAA AUGCAGGU
732	GCAUAUGUU GACUACUA	UAGUAGUC CUGAUGA X GAA ACAUAUGC
737	UGUUGACUA CUACCAGA	UCUGGUAG CUGAUGA X GAA AGUCAACA
740	UGACUACUA CCAGAAAU	AUUUCUGG CUGAUGA X GAA AGUAGUCA
749	CCAGAAAUU AAAGAAAC	GUUUCUUU CUGAUGA X GAA AUUUCUGG
750	CAGAAAUUA AAGAAACC	GGUUUCUU CUGAUGA X GAA AAUUUCUG
766	CAAAAUGUU GGCAUUUU	AAAAUGCC CUGAUGA X GAA ACAUUUUG
772	GUUGGCAUU UUGGUCCG	CGGAO~AA CUGAUGA X GAA AUGCCAAC
773	UUGGCAUUU UGGUCCGC	GCGGACCA CUGAUGA X GAA AAUGCCAA
774	UGGCAUUUU GGUCCGCU	AGCGGACC CUGAUGA X GAA AAAUGCCA
778	AUUUUGGUC CGCUCUCU	AGAGAGCG CUGAUGA A GAA ACCAAAAU
783	GGUCCGCUC UCUCAUUU	AAAUGAGA CUGAUGA X GAA AGCGGACC
785	UCCGCUCUC UCAUUUUG	CAAAAUGA CUGAUGA X GAA AGAGCGGA
787	CGCUCUCUC AUUUUGCA	UGCAAAAU CUGAUGA X GAA AGAGAGCG
790	UCUCUCAUU UUGCAUCC	GGAUGCAA CUGAUGA X GAA AUGAGAGA
791	CUCUCAUUU UGCAUCCA	UGGAUGCA CUGAUGA X GAA AAUGAGAG
792	UCUCAUUUU GCAUCCAA	UUGGAUGC CUGAUGA X GAA AAAUGAGA
797	UUUUGCAUC CAAAUCCG	CGGAUUUG CUGAUGA X GAA AUGCAAAA
803	AUCCAAAUC CGUAGUAA	UUACUACG CUGAUGA X GAA AUUUGGAU
807	AAAUCCGUA GUAAGGAA	UUCCUUAC CUGAUGA X GAA ACGGAUUU
810	UCCGUAGUA AGGAACUA	UAGUUCCU CUGAUGA X GAA ACUACGGA
818	AAGGAACUA AUUUCUGA	UCAGAAAU CUGAUGA X GAA AGUUCCUU
821	GAACUAAUU UCUGAGCA	UGCUCAGA CUGAUGA X GAA AUUAGUUC
822	AACUAAUUU CUGAGCAU	AUGCUCAG CUGAUGA X GAA AAUUAGUU
823	ACUAAUUUC UGAGCAUA	UAUGCUCA CUGAUGA X GAA AAAUUAGU
831	CUGAGCAUA ACAACAAU	AUUGUUGU CUGAUGA X GAA AUGCUCAG
845	AAUGAGAUU GUUAUAGA	UCUAUAAC CUGAUGA X GAA AUCUCAUU
848	GAGAUUGUU AUAGAUUG	CAAUCUAU CUGAUGA X GAA ACAAUCUC
849	AGAUUGUUA UAGAUUGG	CCAAUCUA CUGAUGA X GAA AACAAUCU
851	AUUGUUAUA GAUUGGUU	AACCAAUC CUGAUGA X GAA AUAACAAU
855	UUAUAGAUU GGUUGAAU	AUUCAACC CUGAUGA X GAA AUCUAUAA
859	AGAUUGGUU GAAUGCAC	GUGCAUUC CUGAUGA X GAA ACCAAUCU
876	AGAAACCUA AAUCGGUU	AACCGAUU CUGAUGA X GAA AGGUUUCU
880	ACCUAAAUC GGUUCUCU	AGAGAACC CUGAUGA X GAA AUUUAGGU
884	AAAUCGGUU CUCUAUGU	ACAUAGAG CUGAUGA x GAA ACCGAUUU
885	AAUCGGUUC UCUAUGUA	UACAUAGA CUGAUGA X GAA AACCGAUU
887	UCGGUUCUC UAUGUAUC	GAUACAUA CUGAUGA X GAA AGAACCGA
889	GGUUCUCUA UGUAUCUU	AAGAUACA CUGAUGA X GAA AGAGAACC
893	CUCUAUGUA UCUUUCGG	CCGAAAGA CUGAUGA X GAA ACAUAGAG
895	CUAUGUAUC UUUCGGAA	UUCCGAAA CUGAUGA X GAA AUACAUAG
897	AUGUAUCUU UCGGAAGC	GCUUCCGA CUGAUGA X GAA AGAUACAU
898	UGUAUCUUU CGGAAGCA	UGCUUCCG CUGAUGA X GAA AAGAUACA
899	GUAUCUUUC GGAAGCAU	AUGCUUCC CUGAUGA X GAA AAAGAUAC

Table III

Nt. Position	Substrate	Ribozyme
912	GCAUGGCUA GAUUUCCU	AGGAAAUC CUGAUGA X GAA AGCCAUGC
916	GGCUAGAUU UCCUGAGA	UCUCAGGA CUGAUGA X GAA AUCUAGCC
917	GCUAGAUUU CCUGAGAG	CUCUCAGG CUGAUGA X GAA AAUCUAGC
918	CUAGAUUUC CUGAGAGC	GCUCUCAG CUGAUGA X GAA AAAUCUAG
941	AAUGAAAUA GCCCAAGC	GCUUGGGC CUGAUGA X GAA AUUUCAUU
951	CCCAAGCUC UGGAUGCU	AGCAUCCA CUGAUGA X GAA AGCUUGGG
960	UGGAUGCUU CAAAUGUU	AACAUUUG CUGAUGA X GAA AGCAUCCA
961	GGAUGCUUC AAAUGUUC	GAACAUUU CUGAUGA X GAA AAGCAUCC
968	UCAAAUGUU CCUUUCAU	AUGAAAGG CUGAUGA X GAA ACAUUUGA
969	CAAAUGUUC CUUUCAUU	AAUGAAAG CUGAUGA X GAA AACAUUUG
972	AUGUUCCUU UCAUUUUU	AAAAAUGA CUGAUGA X GAA AGGAACAU
973	UGUUCCUUU CAUUUUUG	CAAAAAUG CUGAUGA X GAA AAGGAACA
974	GUUCCUUUC AUUUUUGU	ACAAAAAU CUGAUGA X GAA AAAGGAAC
977	CCUUUCAUU UUUGUAUU	AAUACAAA CUGAUGA X GAA AUGAAAGG
978	CUUUCAUUU UUGUAUUG	CAAUACAA CUGAUGA X GAA AAUGAAAG
979	UUUCAUUUU UGUAUUGA	UCAAUACA CUGAUGA X GAA AAAUGAAA
980	UUCAUUUUU GUAUUGAG	CUCAAUAC CUGAUGA X GAA AAAAUGAA
983	AUULJUUGUA UUGAGGCC	GGCCUCAA CUGAUGA X GAA ACAAAAAU
985	UUUUGUAUU GAGGCCUA	UAGGCCUC CUGAUGA X GAA AUACAAAA
993	UGAGGCCUA AUGAAGAA	UUCUUCAU CUGAUGA X GAA AGGCCUCA
1009	AACGGCGUC GUGGUUGC	GCAACCAC CUGAUGA X GAA ACGCCGUU
1015	GUCGUGGUU GCCAGUUG	CAACUGGC CUGAUGA X GAA ACCACGAC
1022	UUGCCAGUU GGUAAUUU	AAAUUACC CUGAUGA X GAA ACUGGCAA
1026	CAGUUGGUA AUUUAGAG	CUCUAAAU CUGAUGA X GAA ACCAACUG
1029	UUGGUAAUU UAGAGGAC	GUCCUCUA CUGAUGA X GAA AUUACCAA
1030	UGGUAAUUU AGAGGACA	UGUCCUCU CUGAUGA X GAA AAUUACCA
1031	GGUAAUUUA GAGGACAA	UUGUCCUC CUGAUGA X GAA AAAUUACC
1044	ACAAGACUA AAAAGGGU	ACCCUUUU CUGAUGA X GAA AGUCUUGU
1053	AAAAGGGUU UGUACAUC	GAUGUACA CUGAUGA X GAA ACCCUUUU
1054	AAAGGGUUU GUACAUCA	UGAUGUAC CUGAUGA X GAA AACCCUUU
1057	GGGUUUGUA CAUCAAAG	CUUUGAUG CUGAUGA X GAA ACAAACCC
1061	UUGUACAUC AAAGGGUG	CACCCUUU CUGAUGA X GAA AUGUACAA
1073	GGGUGGGUC CCACAGCU	AGCUGUGG CUGAUGA X GAA ACCCACCC
1082	CCACAGCUU ACGAUCAU	AUGAUCGU CUGAUGA X GAA AGCUGUGG
1083	CACAGCUUA CGAUCAUG	CAUGAUCG CUGAUGA X GAA AAGCUGUG
1088	CUUACGAUC AUGGAACA	UGUUCCAU CUGAUGA X GAA AUCGUAAG
1098	UGGAACAUU CAGCAACA	UGUUGCUG CUGAUGA X GAA AUGUUCCA
1099	GGAACAUUC AGCAACAG	CUGUUGCU CUGAUGA X GAA AAUGUUCC
1114	AGGCGGGUU CAUGACUC	GAGUCAUG CUGAUGA X GAA ACCCGCCU
1115	GGCGGGUUC AUGACUCA	UGAGUCAU CUGAUGA X GAA AACCCGCC
1122	UCAUGACUC AUUGUGGU	ACCACAAU CUGAUGA X GAA AGUCAUGA
1125	UGACUCAUU GUGGUACU	AGUACCAC CUGAUGA X GAA AUGAGUCA
1131	AUUGUGGUA CUAAUUCG	CGAAUUAG CUGAUGA X GAA ACCACAAU
1134	GUGGUACUA AUUCGGUU	AACCGAAU CUGAUGA X GAA AGUACCAC
1137	GUACUAAUU CGGTJUCUG	CAGAACCG CUGAUGA X GAA AUUAGUAC
1138	UACUAAUUC GGUUCUGG	CCAGAACC CUGAUGA X GAA AAUUAGUA
1142	AAUUCGGUU CUGGAAGC	GCUUCCAG CUGAUGA X GAA ACCGAAUU
1143	AUUCGGUUC UGGAAGCC	GGCUUCCA CUGAUGA X GAA AACCGAAU
1154	GAAGCCAUC ACUUUUGG	CCAAAAGU CUGAUGA X GAA AUGGCUUC
1158	CCAUCACUU UUGGCGUG	CACGCCAA CUGAUGA X GAA AGUGAUGG
1159	CAUCACUUU UGGCGUGC	GCACGCCA CUGAUGA X GAA AAGUGAUO
1160	AUCACUUUU GGCGUGCC	GGCACGCC CUGAUGA X GAA AAAGUGAU
1175	CCAAUGAUA ACAUGGCC	GGCCAUGU CUGAUGA X GAA AUCAUUGG
1187	UGGCCACUU UAUGCUGA	UCAGCAUA CUGAUGA X GAA AGUGGCCA
1188	GGCCACUUU AUGCUGAU	AUCAGCAU CUGAUGA X GAA AAGUGGCC
1189	GCCACUUUA UGCUGAUC	GAUCAGCA CUGAUGA X GAA AAAGUGGC
1197	AUGCUGAUC AAUUCUAC	GUAGAAUU CUGAUGA X GAA AUCAGCAU

Table III

Nt. Position	Substrate	Ribozyme
1201	UGAUCAAUU CUACAACG	CGUUGUAG CUGAUGA X GAA AUUGAUCA
1202	GAUCAAUUC UACAACGA	UCGUUGUA CUGAUGA X GAA AAUUGAUC
1204	UCAAUUCUA CAACGAGA	UCUCGUUG CUGAUGA X GAA AGAAUUGA
1217	GAGAAGGUA GUCGAGGU	ACCUCGAC CUGAUGA X GAA ACCUUCUC
1220	AAGGUAGUC GAGGUUAG	CUAACCUC CUGAUGA X GAA ACUACCUU
1226	GUCGAGGUU AGGGGAUU	AAUCCCCU CUGAUGA X GAA ACCUCGAC
1227	UCGAGGUUA GGGGAUUG	CAAUCCCC CUGAUGA X GAA AACCUCGA
1234	UAGGGGAUU GGGAAUCA	UGAUUCCC CUGAUGA X GAA AUCCCCUA
1241	UUGGGAAUC AAAAUCGG	CCGAUUUU CUGAUGA X GAA AUUCCCAA
1247	AUCAAAAUC GGGAUAGA	UCUAUCCC CUGAUGA X GAA AUUUUGAU
1253	AUCGGGAUA GAUGUAUG	CAUACAUC CUGAUGA X GAA AUCCCGAU
1259	AUAGAUGUA UGGAAUGA	UCAUUCCA CUGAUGA X GAA ACAUCUAU
1274	GAAGGGAUU GAGAUCAC	GUGAUCUC CUGAUGA X GAA AUCCCUUC
1280	AUUGAGAUC ACGGGCCC	GGGCCCGU CUGAUGA X GAA AUCUCAAU
1292	GGCCCUGUA AUAGAAAG	CUUUCUAU CUGAUGA X GAA ACAGGGCC
1295	CCUGUAAUA GAAAGCGC	GCGCUUUC CUGAUGA X GAA AUUACAGG
1310	GCCAAGAUU AGAGAAGC	GCUUCUCU CUGAUGA X GAA AUCUUGGC
1311	CCAAGAUUA GAGAAGCA	UGCUUCUC CUGAUGA X GAA AAUCUUGG
1322	GAAGCAAUU GAGAGACU	AGUCUCUC CUGAUGA X GAA AUUGCUUC
1331	GAGAGACUA AUGAUCAG	CUGAUCAU CUGAUGA X GAA AGUCUCUC
1337	CUAAUGAUC AGUAAUGG	CCAUUACU CUGAUGA X GAA AUCAUUAG
1341	UGAUCAGUA AUGGUUCU	AGAACCAU CUGAUGA X GAA ACUGAUCA
1347	GUAAUGGUU CUGAGGAA	UUCCUCAG CUGAUGA X GAA ACCAUUAC
1348	UAAUGGUUC UGAGGAAA	UUUCCUCA CUGAUGA X GAA AACCAUUA
1358	GAGGAAAUU AUAAAUAU	AUAUUUAU CUGAUGA X GAA AUUUCCUC
1359	AGGAAAUUA UAAAUAUU	AAUAUUUA CUGAUGA X GAA AAUUUCCU
1361	GAAAUUAUA AAUAUUAG	CUAAUAUU CUGAUGA X GAA AUAAUUUC
1365	UUAUAAAUA UUAGGGAU	AUCCCUAA CUGAUGA X GAA AUUUAUAA
1367	AUAAAUAUU AGGGAUAG	CUAUCCCU CUGAUGA X GAA AUAUUUAU
1368	UAAAUAUUA GGGAUAGA	UCUAUCCC CUGAUGA X GAA AAUAUUUA
1374	UUAGGGAUA GAGUAAUG	CAUUACUC CUGAUGA X GAA AUCCCUAA
1379	GAUAGAGUA AUGGCUAU	AUAGCCAU CUGAUGA X GAA ACUCUAUC
1386	UAAUGGCUA UGAGCAAA	UUUGCUCA CUGAUGA X GAA AGCCAUUA
1401	AAAUGGCUC AGAAUGCA	UGCAUUCU CUGAUGA X GAA AGCCAUUU
1426	AGGUGGAUC UUCGUGGA	UCCACGAA CUGAUGA X GAA AUCCACCU
1428	GUGGAUCUU CGUGGAAC	GUUCCACG CUGAUGA X GAA AGAUCCAC
1429	UGGAUCUUC GUGGAACA	UGUUCCAC CUGAUGA X GAA AAGAUCCA
1440	GGAACAAUC UCACUGCU	AGCAGUGA CUGAUGA X GAA AUUGUUCC
1442	AACAAUCUC ACUGCUCU	AGAGCAGU CUGAUGA X GAA AGAUUGUU
1449	UCACUGCUC UCAUUCAA	UUGAAUGA CUGAUGA X GAA AGCAGUGA
1451	ACUGCUCUC AUUCAACA	UGUUGAAU CUGAUGA X GAA AGAGCAGU
1454	GCUCUCAUU CAACAUAU	AUAUGUUG CUGAUGA X GAA AUGAGAGC
1455	CUCUCAUUC AACAUAUC	GAUAUGUU CUGAUGA X GAA AAUGAGAG
1461	UUCAACAUA UCAAGAAU	AUUCUUGA CUGAUGA X GAA AUGUUGAA
1463	CAACAUAUC AAGAAUUA	UAAUUCUU CUGAUGA X GAA AUAUGUUG
1470	UCAAGAAUU AUAAUCUU	AAGAUUAU CUGAUGA X GAA AUUCUUGA
1471	CAAGAAUUA UAAUCUUA	UAAGAUUA CUGAUGA X GAA AAUUCUUG
1473	AGAAUUAUA AUCUUAAU	AUUAAGAU CUGAUGA X GAA AUAAUUCU
1476	AUUAUAAUC UUAAUUAG	CUAAUUAA CUGAUGA X GAA AUUAUAAU
1478	UAUAAUCUU AAUUAGUU	AACUAAUU CUGAUGA X GAA AGAUUAUA
1479	AUAAUCUUA AUUAGUUG	CAACUAAU CUGAUGA X GAA AAGAUUAU
1482	AUCUUAAUU AGUUGAAG	CUUCAACU CUGAUGA X GAA AUUAAGAU
1483	UCUUAAUUA GUUGAAGA	UCUUCAAC CUGAUGA X GAA AAUUAAGA
1486	UAAUUAGUU GAAGACAG	CUGUCUUC CUGAUGA X GAA ACUAAUUA
1499	ACAGAAAUA AGUCCUUG	CAAGGACU CUGAUGA X GAA AUUUCUGU
1503	AAAUAAGUC CUUGCAUU	AAUGCAAG CUGAUGA X GAA ACUUAUUU
1506	UAAGUCCUU GCAUUGUA	UACAAUGC CUGAUGA X GAA AGGACUUA

Table III

Nt. Position	Substrate	Ribozyme
1511	CCUUGCAUU GUAACAUG	CAUGUUAC CUGAUGA X GAA AUGCAAGG
1514	UGCAUUGUA ACAUGGUG	CACCAUGU CUGAUGA X GAA ACAAUGCA
1534	GUGUGUGUU UUUUUUCC	GGAAAAAA CUGAUGA X GAA ACACACAC
1535	UGUGUGUUU UUUUUCCA	UGGAAAAA CUGAUGA X GAA AACACACA
1536	GUGUGUUUU UUUUCCAC	GUGGAAAA CUGAUGA X GAA AAACACAC
1537	UGUGUUUUU UUUCCACU	AGUGGAAA CUGAUGA X GAA AAAACACA
1538	GUGUUUUUU UUCCACUU	AAGUGGAA CUGAUGA X GAA AAAAACAC
1539	UGUUUUUUU UCCACUUA	UAAGUGGA CUGAUGA X GAA AAAAAACA
1540	GUUUUUUUU CCACUUAA	UUAAGUGG CUGAUGA X GAA AAAAAAAC
1541	UUUUUUUUC CACUUAAU	AUUAAGUG CUGAUGA X GAA AAAAAAAA
1546	UUUCCACUU AAUAAAAU	AUUUUAUU CUGAUGA X GAA AGUGGAAA
1547	UUCCACUUA AUAAAAUG	CAUUUUAU CUGAUGA X GAA AAGUGGAA
1550	CACUUAAUA AAAUGAAG	CUUCAUUU CUGAUGA X GAA AUUAAGUG
1579	GGAUGGAUC UUAACUUU	AAAGUUAA CUGAUGA X GAA AUCCAUCC
1581	AUGGAUCUU AACUUUAA	UUAAAGUU CUGAUGA X GAA AGAUCCAU
1582	UGGAUCUUA ACUUUAAA	UUUAAAGU CUGAUGA X GAA AAGAUCCA
1586	UCUUAACUU UAAAAAAA	UUUUUUUA CUGAUGA X GAA AGUUAAGA
1587	CUUAACUUU AAAAAAAA	UUUUUUUU CUGAUGA X GAA AAGUUAAG
1588	UUAACUUUA AAAAAAAA	UUUUUUUU CUGAUGA X GAA AAAGUUAA

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be $\geq$ 2 base-pairs.

Table IV: Solanidine glucosyltransferase Hairpin Ribozyme and Target Sequences

79	AUGACC AGAA GAUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UAUCC GCU GGUCAU
211	UGGGAA AGAA GGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUCCG GAU UUCCCA
249	AACUUC AGAA GAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCUCU GCU GAAGUU
376	AAUCAG AGAA GAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACUCC GUC CUGAUU
381	AAUGCA AGAA GGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GUCCU GAU UGCAUU
429	AAGCUC AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUGCU GAU GAGCUU
468	CAUGUA AGAA GACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGUCU GCU UACAUG
511	UGUGAG AGAA GUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUACA GAC CUCACA
524	AGAUUA AGAA GCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAGCA GCC UAAUCU
570	UAUCUC AGAA GGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UACCU GAU GAGAUA
603	CAGAUC AGAA GUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGACA GAU GAUCUG
621	UUGGUC AGAA GACU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGUCG GAU GACCAA
636	GUCAAA AGAA GUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGACU GUU UUUGAC
779	UGAGAG AGAA GACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGUCC GCU CUCUCA
881	AUAGAG AGAA GAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAUCG GUU CUCUAU
1019	AUUACC AGAA GGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGCCA GUU GGUAAU
1078	AUCGUA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACA GCU UACGAU
1139	UUCCAG AGAA GAAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUUCG GUU CUGGAA
1193	GAAUUG AGAA GCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUGCU GAU CAAUUC
1445	AAUGAG AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACU GCU CUCAUU

Table V

Table V: Potato Citrate Synthase Hammerhead Ribozyme and Target Sequences

Nt. Position	Substrate	Ribozyme
9	UUUUUCGUU CCAUCAGC	GCUGAUGG CUGAUGA X GAA ACGAAAAA
10	UUUUCGUUC CAUCAGCC	GGCUGAUG CUGAUGA X GAA AACGAAAA
14	CGUUCCAUC AGCCUACU	AGUAGGCU CUGAUGA X GAA AUGGAACG
20	AUCAGCCUA CUUGAGAU	AUCUCAAG CUGAUGA X GAA AGGCUGAU
23	AGCCUACUU GAGAUGUA	UACAUCUC CUGAUGA X GAA AGUAGGCU
31	UGAGAUGUA UUCCCACU	AGUGGGAA CUGAUGA X GAA ACAUCUCA
33	AGAUGUAUU CCCACUGG	CCAGUGGG CUGAUGA X GAA AUACAUCU
34	GAUGUAUUC CCACUGGU	ACCAGUGG CUGAUGA X GAA AAUACAUC
43	CCACUGGUA AAAGUUAA	UUAACUUU CUGAUGA X GAA ACCAGUGG
49	GUAAAAGUU AAUUUUUU	AAAAAAUU CUGAUGA X GAA ACUUUUAC
50	UAAAAGUUA AUUUUUUU	AAAAAAAU CUGAUGA X GAA AACUUUUA
53	AAGUUAAUU UUUUUGAU	AUCAAAAA CUGAUGA X GAA AUUAACUU
54	AGUUAAUUU UUUUGAUU	AAUCAAAA CUGAUGA X GAA AAUUAACU
55	GUUAAUUUU UUUGAUUU	AAAUCAAA CUGAUGA X GAA AAAUUAAC
56	UUAAUUUUU UUGAUUUU	AAAAUCAA CUGAUGA X GAA AAAAUUAA
57	UAAUUUUUU UGAUUUUC	GAAAAUCA CUGAUGA X GAA AAAAAUUA
58	AAUUUUUUU GAUUUUCG	CGAAAAUC CUGAUGA X GAA AAAAAAUU
62	UUUUUGAUU UUCGCGAG	CUCGCGAA CUGAUGA X GAA AUCAAAAA
63	UUUUGAUUU UCGCGAGC	GCUCGCGA CUGAUGA X GAA AAUCAAAA
64	UUUGAUUUU CGCGAGCA	UGCUCGCG CUGAUGA X GAA AAAUCAAA
65	UUGAUUUUC GCGAGCAA	UUGCUCGC CUGAUGA X GAA AAAAUCAA
80	AAUGGUGUU CUACCGUA	UACGGUAG CUGAUGA X GAA ACACCAUU
81	AUGGUGUUC UACCGUAG	CUACGGUA CUGAUGA X GAA AACACCAU
83	GGUGUUCUA CCGUAGCG	CGCUACGG CUGAUGA X GAA AGAACACC
88	UCUACCGUA GCGUUUCG	CGAAACGC CUGAUGA X GAA ACGGUAGA
93	CGUAGCGUU UCGUUGCU	AGCAACGA CUGAUGA X GAA ACGCUACG
94	GUAGCGUUU CGUUGCUG	CAGCAACG CUGAUGA X GAA AACGCUAC
95	UAGCGUUUC GUUGCUGU	ACAGCAAC CUGAUGA X GAA AAACGCUA
98	CGUUUCGUU GCUGUCAA	UUGACAGC CUGAUGA X GAA ACGAAACG
104	GUUGCUGUC AAAGCUCC	GGAGCUUU CUGAUGA X GAA ACAGCAAC
111	UCAAAGCUC CGCUCUCG	CGAGAGCG CUGAUGA X GAA AGCUUUGA
116	GCUCCGCUC UCGAGCGG	CCGCUCGA CUGAUGA X GAA AGCGGAGC
118	UCCGCUCUC GAGCGGUC	GACCGCUC CUGAUGA X GAA AGAGCGGA
126	CGAGCGGUC CAACAGUC	GACUGUUG CUGAUGA X GAA ACCGCUCG
134	CCAACAGUC AAAUGUUA	UAACAUUU CUGAUGA X GAA ACUGUUGG
141	UCAAAUGUU AGCAAUUC	GAAUUGCU CUGAUGA X GAA ACAUUUGA
142	CAAAUGUUA GCAAUUCU	AGAAUUGC CUGAUGA X GAA AACAUUUG
148	UUAGCAAUU CUGUGCGC	GCGCACAG CUGAUGA X GAA AUUGCUAA
149	UAGCAAUUC UGUGCGCU	AGCGCACA CUGAUGA X GAA AAUUGCUA
162	CGCUGGCUU CAAGUCCA	UGGACUUG CUGAUGA X GAA AGCCAGCG
163	GCUGGCUUC AAGUCCAA	UUGGACUU CUGAUGA X GAA AAGCCAGC
168	CUUCAAGUC CAAACCUC	GAGGUUUG CUGAUGA X GAA ACUUGAAG
176	CCAAACCUC UUCCGGUC	GACCGGAA CUGAUGA X GAA AGGUUUGG
178	AAACCUCUU CCGGUCUU	AAGACCGG CUGAUGA X GAA AGAGGUUU
179	AACCUCUUC CGGUCUUG	CAAGACCG CUGAUGA X GAA AAGAGGUU
184	CUUCCGGUC UUGAUCUG	CAGAUCAA CUGAUGA X GAA ACCGGAAG
186	UCCGGUCUU GAUCUGCG	CGCAGAUC CUGAUGA X GAA AGACCGGA
190	GUCUUGAUC UGCGUUCU	AGAACGCA CUGAUGA X GAA AUCAAGAC
196	AUCUGCGUU CUGAGCUG	CAGCUCAG CUGAUGA X GAA ACGCAGAU
197	UCUGCGUUC UGAGCUGG	CCAGCUCA CUGAUGA X GAA AACGCAGA
207	GAGCUGGUA CAAGAAUU	AAUUCUUG CUGAUGA X GAA ACCAGCUC

Table V

Nt. Position	Substrate	Ribozyme
215	ACAAGAAUU GAUUCCUG	CAGGAAUC CUGAUGA X GAA AUUCUUGU
219	GAAUUGAUU CCUGAACA	UGUUCAGG CUGAUGA X GAA AUCAAUUC
220	AAUUGAUUC CUGAACAA	UUGUUCAG CUGAUGA X GAA AAUCAAUU
235	AACAGGAUC GCCUGAAA	UUUCAGGC CUGAUGA X GAA AUCCUGUU
249	AAAAAGAUC AAGUCAGA	UCUGACUU CUGAUGA X GAA AUCUUUUU
254	GAUCAAGUC AGAUAUGA	UCAUAUCU CUGAUGA X GAA ACUUGAUC
259	AGUCAGAUA UGAAAGGU	ACCUUUCA CUGAUGA X GAA AUCUGACU
268	UGAAAGGUU CAAUUGGG	CCCAAUUG CUGAUGA X GAA ACCUUUCA
269	GAAAGGUUC AAUUGGGA	UCCCAAUU CUGAUGA X GAA AACCUUUC
273	GGUUCAAUU GGGAACAU	AUGUUCCC CUGAUGA X GAA AUUGAACC
282	GGGAACAUC ACAGUUGA	UCAACUGU CUGAUGA X GAA AUGUUCCC
288	AUCACAGUU GAUAUGGU	ACCAUAUC CUGAUGA X GAA ACUGUGAU
292	CAGUUGAUA UGGUUCUU	AAGAACCA CUGAUGA X GAA AUCAACUG
297	GAUAUGGUU CUUGGUGG	CCACCAAG CUGAUGA X GAA ACCAUAUC
298	AUAUGGUUC UUGGUGGA	UCCACCAA CUGAUGA X GAA AACCAUAU
300	AUGGUUCUU GGUGGAAU	AUUCCACC CUGAUGA X GAA AGAACCAU
326	GACAGGAUU ACUGUGGA	UCCACAGU CUGAUGA X GAA AUCCUGUC
327	ACAGGAUUA CUGUGGAA	UUCCACAG CUGAUGA X GAA AAUCCUGU
340	GGAAACCUC AUUACCUU	AAGGUAAU CUGAUGA X GAA AGGUUUCC
343	AACCUCAUU ACCUUGAC	GUCAAGGU CUGAUGA X GAA AUGAGGUU
344	ACCUCAUUA CCUUGACC	GGUCAAGG CUGAUGA X GAA AAUGAGGU
348	CAUUACCUU GACCCUGA	UCAGGGUC CUGAUGA X GAA AGGUAAUG
366	GAGGGAAUU CGCUUCCG	CGGAAGCG CUGAUGA X GAA AUUCCCUC
367	AGGGAAUUC GCUUCCGG	CCGGAAGC CUGAUGA X GAA AAUUCCCU
371	AAUUCGCUU CCGGGGGU	ACCCCCGG CUGAUGA X GAA AGCGAAUU
372	AUUCGCUUC CGGGGGUU	AACCCCCG CUGAUGA X GAA AAGCGAAU
380	CCGGGGGUU GUCUAUAC	GUAUAGAC CUGAUGA X GAA ACCCCCGG
383	GGGGUUGUC UAUACCUG	CAGGUAUA CUGAUGA X GAA ACAACCCC
385	GGUUGUCUA UACCUGAA	UUCAGGUA CUGAUGA X GAA AGACAACC
387	UUGUCUAUA CCUGAAUG	CAUUCAGG CUGAUGA X GAA AUAGACAA
405	CAAAAGGUA UUACCUGC	GCAGGUAA CUGAUGA X GAA ACCUUUUG
407	AAAGGUAUU ACCUGCAG	CUGCAGGU CUGAUGA X GAA AUACCUUU
408	AAGGUAUUA CCUGCAGC	GCUGCAGG CUGAUGA X GAA AAUACCUU
437	UGAGCCCUU GCCUGAAG	CUUCAGGC CUGAUGA X GAA AGGGCUCA
448	CUGAAGGUC UUCUCUGG	CCAGAGAA CUGAUGA X GAA ACCUUCAG
450	GAAGGUCUU CUCUGGCU	AGCCAGAG CUGAUGA X GAA AGACCUUC
451	AAGGUCUUC UCUGGCUU	AAGCCAGA CUGAUGA X GAA AAGACCUU
453	GGUCUUCUC UGGCUUCU	AGAAGCCA CUGAUGA X GAA AGAAGACC
459	CUCUGGCUU CUUUUAAC	GUUAAAAG CUGAUGA X GAA AGCCAGAG
460	UCUGGCUUC UUUUAACA	UGUUAAAA CUGAUGA X GAA AAGCCAGA
462	UGGCUUCUU UUAACAGG	CCUGUUAA CUGAUGA X GAA AGAAGCCA
463	GGCUUCUUU UAACAGGA	UCCUGUUA CUGAUGA X GAA AAGAAGCC
464	GCUUCUUUU AACAGGAA	UUCCUGUU CUGAUGA X GAA AAAGAAGC
465	CUUCUUUUA ACAGGAAA	UUUCCUGU CUGAUGA X GAA AAAAGAAG
482	GGUGCCAUC AAAAGAGC	GCUCUUUU CUGAUGA X GAA AUGGCACC
499	AAGUGAAUU CAAUUGUC	GACAAUUG CUGAUGA X GAA AUUCACUU
500	AGUGAAUUC AAUUGUCU	AGACAAUU CUGAUGA X GAA AAUUCACU
504	AAUUCAAUU GUCUCAGG	CCUGAGAC CUGAUGA X GAA AUUGAAUU
507	UCAAUUGUC UCAGGAAU	AUUCCUGA CUGAUGA X GAA ACAAUUGA
509	AAUUGUCUC AGGAAUUG	CAAUUCCU CUGAUGA X GAA AGACAAUU
516	UCAGGAAUU GCAGAGUC	GACUCUGC CUGAUGA X GAA AUUCCUGA
524	UGCAGAGUC GGGCAUCA	UGAUGCCC CUGAUGA X GAA ACUCUGCA
531	UCGGGCAUC AUAUCCCU	AGGGAUAU CUGAUGA X GAA AUGCCCGA
534	GGCAUCAUA UCCCUGAU	AUCAGGGA CUGAUGA X GAA AUGAUGCC
536	CAUCAUAUC CCUGAUCA	UGAUCAGG CUGAUGA X GAA AUAUGAUG
543	UCCCUGAUC AUCAUGUA	UACAUGAU CUGAUGA X GAA AUCAGGGA
546	CUGAUCAUC AUGUAUAC	GUAUACAU CUGAUGA X GAA AUGAUCAG

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Nt. Position	Substrate	Ribozyme
551	CAUCAUGUA UACAACUA	UAGUUGUA CUGAUGA X GAA ACAUGAUG
553	UCAUGUAUA CAACUAUU	AAUAGUUG CUGAUGA X GAA AUACAUGA
559	AUACAACUA UUGAUGCC	GGCAUCAA CUGAUGA X GAA AGUUGUAU
561	ACAACUAUU GAUGCCUU	AAGGCAUC CUGAUGA X GAA AUAGUUGU
569	UGAUGCCUU ACCAGUCA	UGACUGGU CUGAUGA X GAA AGGCAUCA
570	GAUGCCUUA CCAGUCAC	GUGACUGG CUGAUGA X GAA AAGGCAUC
576	UUACCAGUC ACAGCUCA	UGAGCUGU CUGAUGA X GAA ACUGGUAA
583	UCACAGCUC AUCCAAUG	CAUUGGAU CUGAUGA X GAA AGCUGUGA
586	CAGCUCAUC CAAUGACC	GGUCAUUG CUGAUGA X GAA AUGAGCUG
599	GACCCAGUU UGCUACUG	CAGUAGCA CUGAUGA X GAA ACUGGGUC
600	ACCCAGUUU GCUACUGG	CCAGUAGC CUGAUGA X GAA AACUGGGU
604	AGUUUGCUA CUGGAGUC	GACUCCAG CUGAUGA X GAA AGCAAACU
612	ACUGGAGUC AUGGCUCU	AGAGCCAU CUGAUGA X GAA ACUCCAGU
619	UCAUGGCUC UUCAGGUU	AACCUGAA CUGAUGA X GAA AGCCAUGA
621	AUGGCUCUU CAGGUUCA	UGAACCUG CUGAUGA X GAA AGAGCCAU
622	UGGCUCUUC AGGUUCAA	UUGAACCU CUGAUGA X GAA AAGAGCCA
627	CUUCAGGUU CAAAGUGA	UCACUUUG CUGAUGA X GAA ACCUGAAG
628	UUCAGGUUC AAAGUGAA	UUCACUUU CUGAUGA X GAA AACCUGAA
638	AAGUGAAUU UCAAAAGG	CCUUUUGA CUGAUGA X GAA AUUCACUU
639	AGUGAAUUU CAAAAGGC	GCCUUUUG CUGAUGA X GAA AAUUCACU
640	GUGAAUUUC AAAAGGCA	UGCCUUUU CUGAUGA X GAA AAAUUCAC
650	AAAGGCAUA CGAGAAAG	CUUUCUCG CUGAUGA X GAA AUGCCUUU
663	AAAGGGAUU CACAAAUC	GAUUUGUG CUGAUGA X GAA AUCCCUUU
664	AAGGGAUUC ACAAAUCA	UGAUUUGU CUGAUGA X GAA AAUCCCUU
671	UCACAAAUC AAAGUAUU	AAUACUUU CUGAUGA X GAA AUUUGUGA
677	AUCAAAGUA UUGGGAAC	GUUCCCAA CUGAUGA X GAA ACUUUGAU
679	CAAAGUAUU GGGAACCA	UGGUUCCC CUGAUGA X GAA AUACUUUG
692	ACCAACAUA UGAGGAUU	AAUCCUCA CUGAUGA X GAA AUGUUGGU
700	AUGAGGAUU CCAUGAAU	AUUCAUGG CUGAUGA X GAA AUCCUCAU
701	UGAGGAUUC CAUGAAUC	GAUUCAUG CUGAUGA X GAA AAUCCUCA
709	CCAUGAAUC UGAUUGCU	AGCAAUCA CUGAUGA X GAA AUUCAUGG
714	AAUCUGAUU GCUCAAGU	ACUUGAGC CUGAUGA X GAA AUCAGAUU
718	UGAUUGCUC AAGUUCCA	UGGAACUU CUGAUGA X GAA AGCAAUCA
723	GCUCAAGUU CCACUUGU	ACAAGUGG CUGAUGA X GAA ACUUGAGC
724	CUCAAGUUC CACUUGUU	AACAAGUG CUGAUGA X GAA AACUUGAG
729	GUUCCACUU GUUGCUGC	GCAGCAAC CUGAUGA X GAA AGUGGAAC
732	CCACUUGUU GCUGCUUA	UAAGCAGC CUGAUGA X GAA ACAAGUGG
739	UUGCUGCUU AUGUUUAU	AUAAACAU CUGAUGA X GAA AGCAGCAA
740	UGCUGCUUA UGUUUAUC	GAUAAACA CUGAUGA X GAA AAGCAGCA
744	GCUUAUGUU UAUCGCAG	CUGCGAUA CUGAUGA X GAA ACAUAAGC
745	CUUAUGUUU AUCGCAGG	CCUGCGAU CUGAUGA X GAA AACAUAAG
746	UUAUGUUUA UCGCAGGA	UCCUGCGA CUGAUGA X GAA AAACAUAA
748	AUGUUUAUC GCAGGAUG	CAUCCUGC CUGAUGA X GAA AUAAACAU
758	CAGGAUGUA CAAGAAUG	CAUUCUUG CUGAUGA X GAA ACAUCCUG
775	GUGACACUA UACCUAAG	CUUAGGUA CUGAUGA X GAA AGUGUCAC
777	GACACUAUA CCUAAGGA	UCCUUAGG CUGAUGA X GAA AUAGUGUC
781	CUAUACCUA AGGAUGAA	UUCAUCCU CUGAUGA X GAA AGGUAUAG
791	GGAUGAAUC CCUGGAUU	AAUCCAGG CUGAUGA X GAA AUUCAUCC
799	CCCUGGAUU AUGGUGCA	UGCACCAU CUGAUGA X GAA AUCCAGGG
800	CCUGGAUUA UGGUGCAA	UUGCACCA CUGAUGA X GAA AAUCCAGG
811	GUGCAAAUU UUGCUCAC	GUGAGCAA CUGAUGA X GAA AUUUGCAC
812	UGCAAAUUU UGCUCACA	UGUGAGCA CUGAUGA X GAA AAUUUGCA
813	GCAAAUUUU GCUCACAU	AUGUGAGC CUGAUGA X GAA AAAUUUGC
817	AUUUUGCUC ACAUGCUU	AAGCAUGU CUGAUGA X GAA AGCAAAAU
825	CACAUGCUU GGUUUCAG	CUGAAACC CUGAUGA X GAA AGCAUGUG
829	UGCUUGGUU UCAGUAGC	GCUACUGA CUGAUGA X GAA ACCAAGCA
830	GCUUGGUUU CAGUAGCU	AGCUACUG CUGAUGA X GAA AACCAAGC

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Nt. Position	Substrate	Ribozyme
831	CUUGGUUUC AGUAGCUC	GAGCUACU CUGAUGA X GAA AAACCAAG
835	GUUUCAGUA GCUCUGAA	UUCAGAGC CUGAUGA X GAA ACUGAAAC
839	CAGUAGCUC UGAAAUGC	GCAUUUCA CUGAUGA X GAA AGCUACUG
855	CAUGAACUU CUUAUGAG	CUCAUAAG CUGAUGA X GAA AGUUCAUG
856	AUGAACUUC UUAUGAGG	CCUCAUAA CUGAUGA X GAA AAGUUCAU
858	GAACUUCUU AUGAGGCU	AGCCUCAU CUGAUGA X GAA AGAAGUUC
859	AACUUCUUA UGAGGCUC	GAGCCUCA CUGAUGA X GAA AAGAAGUU
867	AUGAGGCUC UAUGUAAC	GUUACAUA CUGAUGA X GAA AGCCUCAU
869	GAGGCUCUA UGUAACAA	UUGUUACA CUGAUGA X GAA AGAGCCUC
873	CUCUAUGUA ACAAUACA	UGUAUUGU CUGAUGA X GAA ACAUAGAG
879	GUAACAAUA CACAGUGA	UCACUGUG CUGAUGA X GAA AUUGUUAC
889	ACAGUGAUC AUGAAGGU	ACCUUCAU CUGAUGA X GAA AUCACUGU
901	AAGGUGGUA AUGUCAGU	ACUGACAU CUGAUGA X GAA ACCACCUU
906	GGUAAUGUC AGUGCUCA	UGAGCACU CUGAUGA X GAA ACAUUACC
913	UCAGUGCUC ACACCGGU	ACCGGUGU CUGAUGA X GAA AGCACUGA
922	ACACCGGUC ACUUGGUU	AACCAAGU CUGAUGA X GAA ACCGGUGU
926	CGGUCACUU GGUUGCUA	UAGCAACC CUGAUGA X GAA AGUGACCG
930	CACUUGGUU GCUAGUGC	GCACUAGC CUGAUGA X GAA ACCAAGUG
934	UGGUUGCUA GUGCUUUG	CAAAGCAC CUGAUGA X GAA AGCAACCA
940	CUAGUGCUU UGUCUGAU	AUCAGACA CUGAUGA X GAA AGCACUAG
941	UAGUGCUUU GUCUGAUC	GAUCAGAC CUGAUGA X GAA AAGCACUA
944	UGCUUUGUC UGAUCCUU	AAGGAUCA CUGAUGA X GAA ACAAAGCA
949	UGUCUGAUC CUUACCUC	GAGGUAAG CUGAUGA X GAA AUCAGACA
952	CUGAUCCUU ACCUCUCC	GGAGAGGU CUGAUGA X GAA AGGAUCAG
953	UGAUCCUUA CCUCUCCU	AGGAGAGG CUGAUGA X GAA AAGGAUCA
957	CCUUACCUC UCCUUUGC	GCAAAGGA CUGAUGA X GAA AGGUAAGG
959	UUACCUCUC CUUUGCUG	CAGCAAAG CUGAUGA X GAA AGAGGUAA
962	CCUCUCCUU UGCUGCUG	CAGCAGCA CUGAUGA X GAA AGGAGAGG
963	CUCUCCUUU GCUGCUGC	GCAGCAGC CUGAUGA X GAA AAGGAGAG
973	CUGCUGCUU UGAAUGGU	ACCAUUCA CUGAUGA X GAA AGCAGCAG
974	UGCUGCUUU GAAUGGUU	AACCAUUC CUGAUGA X GAA AAGCAGCA
982	UGAAUGGUU UAGCCGGA	UCCGGCUA CUGAUGA X GAA ACCAUUCA
983	GAAUGGUUU AGCCGGAC	GUCCGGCU CUGAUGA X GAA AACCAUUC
984	AAUGGUUUA GCCGGACC	GGUCCGGC CUGAUGA X GAA AAACCAUU
996	GGACCACUU CAUGGUUU	AAACCAUG CUGAUGA X GAA AGUGGUCC
997	GACCACUUC AUGGUUUA	UAAACCAU CUGAUGA X GAA AAGUGGUC
600	UUCAUGGUU UAGCCAAU	AUUGGCUA CUGAUGA X GAA ACCAUGAA
1004	UCAUGGUUU AGCCAAUC	GAUUGGCU CUGAUGA X GAA AACCAUGA
1005	CAUGGUUUA GCCAAUCA	UGAUUGGC CUGAUGA X GAA AAACCAUG
1012	UAGCCAAUC AGGAAGUU	AACUUCCU CUGAUGA X GAA AUUGGCUA
1020	CAGGAAGUU UUGCUAUG	CAUAGCAA CUGAUGA X GAA ACUUCCUG
1021	AGGAAGUUU UGCUAUGG	CCAUAGCA CUGAUGA X GAA AACUUCCU
1022	GGAAGUUUU GCUAUGGA	UCCAUAGC CUGAUGA X GAA AAACUUCC
1026	GUUUUGCUA UGGAUAAA	UUUAUCCA CUGAUGA X GAA AGCAAAAC
1032	CUAUGGAUA AAAUCUGU	ACAGAUUU CUGAUGA X GAA AUCCAUAG
1037	GAUAAAAUC UGUUGUAG	CUACAACA CUGAUGA X GAA AUUUUAUC
1041	AAAUCUGUU GUAGAAGA	UCUUCUAC CUGAUGA X GAA ACAGAUUU
1044	UCUGUUGUA GAAGAAUG	CAUUCUUC CUGAUGA X GAA ACAACAGA
1065	GAGAACAUU UCCAAAGA	UCUUUGGA CUGAUGA X GAA AUGUUCUC
1066	AGAACAUUU CCAAAGAG	CUCUUUGG CUGAUGA X GAA AAUGUUCU
1067	GAACAUUUC CAAAGAGC	GCUCUUUG CUGAUGA X GAA AAAUGUUC
1079	AGAGCAGUU GAAAGACU	AGUCUUUC CUGAUGA X GAA ACUGCUCU
1088	GAAAGACUA UGUUUGGA	UCCAAACA CUGAUGA X GAA AGUCUUUC
1092	GACUAUGUU UGGAAAAC	GUUUUCCA CUGAUGA X GAA ACAUAGUC
1093	ACUAUGUUU GGAAAACA	UGUUUUCC CUGAUGA X GAA AACAUAGU
1103	GAAAACAUU GAACAGUG	CACUGUUC CUGAUGA X GAA AUGUUUUC
1119	GGCAAGGUU GUCCCUGG	CCAGGGAC CUGAUGA X GAA ACCUUGCC

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Nt. Position	Substrate	Ribozyme
1122	AAGGUUGUC CCUGGUUU	AAACCAGG CUGAUGA X GAA ACAACCUU
1129	UCCCUGGUU UUGGACAU	AUGUCCAA CUGAUGA X GAA ACCAGGGA
1130	CCCUGGUUU UGGACAUG	CAUGUCCA CUGAUGA X GAA AACCAGGG
1131	CCUGGUUUU GGACAUGG	CCAUGUCC CUGAUGA X GAA AAACCAGG
1143	CAUGGAGUU CUGCGAAA	UUUCGCAG CUGAUGA X GAA ACUCCAUG
1144	AUGGAGUUC UGCGAAAG	CUUUCGCA CUGAUGA X GAA AACUCCAU
1158	AAGACUGUA CCAAGAUA	UAUCUUGG CUGAUGA X GAA ACAGUCUU
1166	ACCAAGAUA UACAUGCC	GGCAUGUA CUGAUGA X GAA AUCUUGGU
1168	CAAGAUAUA CAUGCCAG	CUGGCAUG CUGAUGA X GAA AUAUCUUG
1184	GAGAGAGUU CGCUAUGA	UCAUAGCG CUGAUGA X GAA ACUCUCUC
1185	AGAGAGUUC GCUAUGAA	UUCAUAGC CUGAUGA X GAA AACUCUCU
1189	AGUUCGCUA UGAAGCAU	AUGCUUCA CUGAUGA X GAA AGCGAACU
1198	UGAAGCAUU UGCCUGAA	UUCAGGCA CUGAUGA X GAA AUGCUUCA
1199	GAAGCAUUU GCCUGAAG	CUUCAGGC CUGAUGA X GAA AAUGCUUC
1210	CUGAAGAUC CACUGUUU	AAACAGUG CUGAUGA X GAA AUCUUCAG
1217	UCCACUGUU UCAACUGG	CCAGUUGA CUGAUGA X GAA ACAGUGGA
1218	CCACUGUUU CAACUGGU	ACCAGUUG CUGAUGA X GAA AACAGUGG
1219	CACUGUUUC AACUGGUU	AACCAGUU CUGAUGA X GAA AAACAGUG
1227	CAACUGGUU UCAAAACU	AGUUUUGA CUGAUGA X GAA ACCAGUUG
1228	AACUGGUUU CAAAACUC	GAGUUUUG CUGAUGA X GAA AACCAGUU
1229	ACUGGUUUC AAAACUCU	AGAGUUUU CUGAUGA X GAA AAACCAGU
1236	UCAAAACUC UACGAAGU	ACUUCGUA CUGAUGA X GAA AGUUUUGA
1238	AAAACUCUA CGAAGUUU	AAACUUCG CUGAUGA X GAA AGAGUUUU
1245	UACGAAGUU UUCCUCCU	AGGAGGAA CUGAUGA X GAA ACUUCGUA
1246	ACGAAGUUU UCCUCCUG	CAGGAGGA CUGAUGA X GAA AACUUCGU
1247	CGAAGUUUU CCUCCUGU	ACAGGAGG CUGAUGA X GAA AAACUUCG
1248	GAAGUUUUC CUCCUGUU	AACAGGAG CUGAUGA X GAA AAAACUUC
1251	GUUUUCCUC CUGUUCUU	AAGAACAG CUGAUGA X GAA AGGAAAAC
1256	CCUCCUGUU CUUACAGA	UCUGUAAG CUGAUGA X GAA ACAGGAGG
1257	CUCCUGUUC UUACAGAA	UUCUGUAA CUGAUGA X GAA AACAGGAG
1259	CCUGUUCUU ACAGAACU	AGUUCUGU CUGAUGA X GAA AGAACAGG
1260	CUGUUCUUA CAGAACUU	AAGUUCUG CUGAUGA X GAA AAGAACAG
1268	ACAGAACUU GGCAAAGU	ACUUUGCC CUGAUGA X GAA AGUUCUGU
1277	GGCAAAGUU AAAACCUU	AAGGUUUU CUGAUGA X GAA ACUUUGCC
1278	GCAAAGUUA AAACCUUG	CAAGGUUU CUGAUGA X GAA AACUUUGC
1285	UAAAACCUU GGCCAAAU	AUUUGGCC CUGAUGA X GAA AGGUUUUA
1296	CCAAAUGUU GAUGCCCA	UGGGCAUC CUGAUGA X GAA ACAUUUGG
1316	UGGUGUGUU GUUGAACU	AGUUCAAC CUGAUGA X GAA ACACACCA
1319	UGUGUUGUU GAACUAUU	AAUAGUUC CUGAUGA X GAA ACAACACA
1325	GUUGAACUA UUAUGGUU	AACCAUAA CUGAUGA X GAA AGUUCAAC
1327	UGAACUAUU AUGGUUUA	UAAACCAU CUGAUGA X GAA AUAGUUCA
1328	GAACUAUUA UGGUUUAA	UUAAACCA CUGAUGA X GAA AAUAGUUC
1333	AUUAUGGUU UAACUGAA	UUCAGUUA CUGAUGA X GAA ACCAUAAU
1334	UUAUGGUUU AACUGAAG	CUUCAGUU CUGAUGA X GAA AACCAUAA
1335	UAUGGUUUA ACUGAAGC	GCUUCAGU CUGAUGA X GAA AAACCAUA
1349	AGCAAGAUA UUAUACGG	CCGUAUAA CUGAUGA X GAA AUCUUGCU
1351	CAAGAUAUU AUACGGUC	GACCGUAU CUGAUGA X GAA AUAUCUUG
1352	AAGAUAUUA UACGGUCC	GGACCGUA CUGAUGA X GAA AAUAUCUU
1354	GAUAUUAUA CGGUCCUC	GAGGACCG CUGAUGA X GAA AUAAUAUC
1359	UAUACGGUC CUCUUUGG	CCAAAGAG CUGAUGA X GAA ACCGUAUA
1362	ACGGUCCUC UUUGGCGU	ACGCCAAA CUGAUGA X GAA AGGACCGU
1364	GGUCCUCUU UGGCGUAU	AUACGCCA CUGAUGA X GAA AGAGGACC
1365	GUCCUCUUU GGCGUAUC	GAUACGCC CUGAUGA X GAA AAGAGGAC
1371	UUUGGCGUA UCAAGAGC	GCUCUUGA CUGAUGA X GAA ACGCCAAA
1373	UGGCGUAUC AAGAGCUC	GAGCUCUU CUGAUGA X GAA AUACGCCA
1381	CAAGAGCUC UUGGCAUU	AAUGCCAA CUGAUGA X GAA AGCUCJUG
1383	AGAGCUCUU GGCAUUUG	CAAAUGCC CUGAUGA X GAA AGAGCUCU

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Nt. Position	Substrate	Ribozyme
1389	CUUGGCAUU UGCUCUCA	UGAGAGCA CUGAUGA X GAA AUGCCAAG
1390	UUGGCAUUU GCUCUCAG	CUGAGAGC CUGAUGA X GAA AAUGCCAA
1394	CAUUUGCUC UCAGCUAA	UUAGCUGA CUGAUGA X GAA AGCAAAUG
1396	UUUGCUCUC AGCUAAUU	AAUUAGCU CUGAUGA X GAA AGAGCAAA
1401	UCUCAGCUA AUUUGGGA	UCCCAAAU CUGAUGA X GAA AGCUGAGA
1404	CAGCUAAUU UGGGACCG	CGGUCCCA CUGAUGA X GAA AUUAGCUG
1405	AGCUAAUUU GGGACCGA	UCGGUCCC CUGAUGA X GAA AAUUAGCU
1417	ACCGAGCUC UUGGAUUG	CAAUCCAA CUGAUGA X GAA AGCUCGGU
1419	CGAGCUCUU GGAUUGCC	GGCAAUCC CUGAUGA X GAA AGAGCUCG
1424	UCUUGGAUU GCCGCUAG	CUAGCGGC CUGAUGA X GAA AUCCAAGA
1431	UUGCCGCUA GAGAGGCC	GGCCUCUC CUGAUGA X GAA AGCGGCAA
1449	AAGAGUGUC ACAAUGGA	UCCAUUGU CUGAUGA X GAA ACACUCUU
1464	GAGUGGCUU GAGAACCA	UGGUUCUC CUGAUGA X GAA AGCCACUC
1491	GCAUGAAUU GUUUGAAA	UUUCAAAC CUGAUGA X GAA AUUCAUGC
1494	UGAAUUGUU UGAAAUCU	AGAUUUCA CUGAUGA X GAA ACAAUUCA
1495	GAAUUGUUU GAAAUCUC	GAGAUUUC CUGAUGA X GAA AACAAUUC
1501	UUUGAAAUC UCGCGAGC	GCUCGCGA CUGAUGA X GAA AUUUCAAA
1503	UGAAAUCUC GCGAGCAU	AUGCUCGC CUGAUGA X GAA AGAUUUCA
1512	GCGAGCAUA AAACACAA	UUGUGUUU CUGAUGA X GAA AUGCUCGC
1524	CACAAUGUA UAAUCUCU	AGAGAUUA CUGAUGA X GAA ACAUUGUG
1526	CAAUGUAUA AUCUCUAU	AUAGAGAU CUGAUGA X GAA AUACAUUG
1529	UGUAUAAUC UCUAUGAA	UUCAUAGA CUGAUGA X GAA AUUAUACA
1531	UAUAAUCUC UAUGAAUA	UAUUCAUA CUGAUGA X GAA AGAUUAUA
1533	UAAUCUCUA UGAAUAAU	AUUAUUCA CUGAUGA X GAA AGAGAUUA
1539	CUAUGAAUA AUUGCUUG	CAAGCAAU CUGAUGA X GAA AUUCAUAG
1542	UGAAUAAUU GCUUGACA	UGUCAAGC CUGAUGA X GAA AUUAUUCA
1546	UAAUUGCUU GACAAAGC	GCUUUGUC CUGAUGA X GAA AGCAAUUA
1558	AAAGCACUC CUUUCUUG	CAAGAAAG CUGAUGA X GAA AGUGCUUU
1561	GCACUCCUU UCUUGGGG	CCCCAAGA CUGAUGA X GAA AGGAGUGC
1562	CACUCCUUU CUUGGGGG	CCCCCAAG CUGAUGA X GAA AAGGAGUG
1563	ACUCCUUUC UUGGGGGA	UCCCCCAA CUGAUGA X GAA AAAGGAGU
1565	UCCUUUCUU GGGGGACA	UGUCCCCC CUGAUGA X GAA AGAAAGGA
1578	GACAAGAUA GGUCGGCC	GGCCGACC CUGAUGA X GAA AUCUUGUC
1582	AGAUAGGUC GGCCCUUC	GAAGGGCC CUGAUGA X GAA ACCUAUCU
1589	UCGGCCCUU CAAUGGGU	ACCCAUUG CUGAUGA X GAA AGGGCCGA
1590	CGGCCCUUC AAUGGGUU	AACCCAUU CUGAUGA X GAA AAGGGCCG
1598	CAAUGGGUU AACGAACU	AGUUCGUU CUGAUGA X GAA ACCCAUUG
1599	AAUGGGUUA ACGAACUU	AAGUUCGU CUGAUGA X GAA AACCCAUU
1607	AACGAACUU CAGUUCAA	UUGAACUG CUGAUGA X GAA AGUUCGUU
1608	ACGAACUUC AGUUCAAA	UUUGAACU CUGAUGA X GAA AAGUUCGU
1612	ACUUCAGUU CAAACUUC	GAAGUUUG CUGAUGA X GAA ACUGAAGU
1613	CUUCAGUUC AAACUUCA	UGAAGUUU CUGAUGA X GAA AACUGAAG
1619	UUCAAACUU CACUGAAU	AUUCAGUG CUGAUGA X GAA AGUUUGAA
1620	UCAAACUUC ACUGAAUU	AAUUCAGU CUGAUGA X GAA AAGUUUGA
1628	CACUGAAUU UGUGUGAA	UUCACACA CUGAUGA X GAA AUUCAGUG
1629	ACUGAAUUU GUGUGAAU	AUUCACAC CUGAUGA X GAA AAUUCAGU
1638	GUGUGAAUU GUAUGGUU	AACCAUAC CUGAUGA X GAA AUUCACAC
1641	UGAAUUGUA UGGUUUCU	AGAAACCA CUGAUGA X GAA ACAAUUCA
1646	UGUAUGGUU UCUCGAGA	UCUCGAGA CUGAUGA X GAA ACCAUACA
1647	GUAUGGUUU CUCGAGAC	GUCUCGAG CUGAUGA X GAA AACCAUAC
1648	UAUGGUUUC UCGAGACU	AGUCUCGA CUGAUGA X GAA AAACCAUA
1650	UGGUUUCUC GAGACUUG	CAAGUCUC CUGAUGA X GAA AGAAACCA
1657	UCGAGACUU GUCCUGAA	UUCAGGAC CUGAUGA X GAA AGUCUCGA
1660	AGACUUGUC CUGAAUUU	AAAUUCAG CUGAUGA X GAA ACAAGUCU
1667	UCCUGAAUU UUGAACUU	AAGUUCAA CUGAUGA X GAA AUUCAGGA
1668	CCUGAAUUU UGAACUUA	UAAGUUCA CUGAUGA X GAA AAUUCAGG
1669	CUGAAUUUU GAACUUAG	CUAAGUUC CUGAUGA X GAA AAAUUCAG

Table V

Nt. Position	Substrate	Ribozyme
1675	UUUGAACUU AGUCUAGU	ACUAGACU CUGAUGA X GAA AGUUCAAA
1676	UUGAACUUA GUCUAGUG	CACUAGAC CUGAUGA X GAA AAGUUCAA
1679	AACUUAGUC UAGUGGAU	AUCCACUA CUGAUGA X GAA ACUAAGUU
1681	CUUAGUCUA GUGGAUUC	GAAUCCAC CUGAUGA X GAA AGACUAAG
1668	UAGUGGAUU CAUUUUUC	GAAAAAUG CUGAUGA X GAA AUCCACUA
1689	AGUGGAUUC AUUUUUCU	AGAAAAAU CUGAUGA X GAA AAUCCACU
1692	GGAUUCAUU UUUCUUCA	UGAAGAAA CUGAUGA X GAA AUGAAUCC
1693	GAUUCAUUU UUCUUCAU	AUGAAGAA CUGAUGA X GAA AAUGAAUC
1694	AUUCAUUUU UCUUCAUU	AAUGAAGA CUGAUGA X GAA AAAUGAAU
1695	UUCAUUUUU CUUCAUUC	GAAUGAAG CUGAUGA X GAA AAAAUGAA
1696	UCAUUUUUC UUCAUUCC	GGAAUGAA CUGAUGA X GAA AAAAAUGA
1698	AUUUUUCUU CAUUCCGA	UCGGAAUG CUGAUGA X GAA AGAAAAAU
1699	UUUUUCUUC AUUCCGAA	UUCGGAAU CUGAUGA X GAA AAGAAAAA
1702	UUCUUCAUU CCGAAUUC	GAAUUCGG CUGAUGA X GAA AUGAAGAA
1703	UCUUCAUUC CGAAUUCC	GGAAUUCG CUGAUGA X GAA AAUGAAGA
1709	UUCCGAAUU CCUCACAC	GUGUGAGG CUGAUGA X GAA AUUCGGAA
1710	UCCGAAUUC CUCACACG	CGUGUGAG CUGAUGA X GAA AAUUCGGA
1713	GAAUUCCUC ACACGCUG	CAGCGUGU CUGAUGA X GAA AGGAAUUC
1724	ACGCUGAUC CAGCAUGU	ACAUGCUG CUGAUGA X GAA AUCAGCGU
1733	CAGCAUGUA AAAAUUAA	UUAAUUUU CUGAUGA X GAA ACAUGCUG
1739	GUAAAAAUU AAUAGGUC	GACCUAUU CUGAUGA X GAA AUUUUUAC
1740	UAAAAAUUA AUAGGUCA	UGACCUAU CUGAUGA X GAA AAUUUUUA
1743	AAAUUAAUA GGUCAAUG	CAUUGACC CUGAUGA X GAA AUUAAUUU
1747	UAAUAGGUC AAUGCUAU	AUAGCAUU CUGAUGA X GAA ACCUAUUA
1754	UCAAUGCUA UUAAUCGC	GCGAUUAA CUGAUGA X GAA AGCAUUGA
1756	AAUGCUAUU AAUCGCGU	ACGCGAUU CUGAUGA X GAA AUAGCAUU
1757	AUGCUAUUA AUCGCGUU	AACGCGAU CUGAUGA X GAA AAUAGCAU
1760	CUAUUAAUC GCGUUCUU	AAGAACGC CUGAUGA X GAA AUUAAUAG
1765	AAUCGCGUU CUUGGUUG	CAACCAAG CUGAUGA X GAA ACGCGAUU
1766	AUCGCGUUC UUGGUUGC	GCAACCAA CUGAUGA X GAA AACGCGAU
1768	CGCGUUCUU GGUUGCCA	UGGCAACC CUGAUGA X GAA AGAACGCG
1772	UUCUUGGUU GCCAUUAG	CUAAUGGC CUGAUGA X GAA ACCAAGAA
1778	GUUGCCAUU AGACUUGU	ACAAGUCU CUGAUGA X GAA AUGGCAAC
1779	UUGCCAUUA GACUUGUG	CACAAGUC CUGAUGA X GAA AAUGGCAA
1784	AUUAGACUU GUGAAUGA	UCAUUCAC CUGAUGA X GAA AGUCUAAU
1795	GAAUGACUU CCUUUGCU	AGCAAAGG CUGAUGA X GAA AGUCAUUC
1796	AAUGACUUC CUUUGCUG	CAGCAAAG CUGAUGA X GAA AAGUCAUU
1799	GACUUCCUU UGCUGGAA	UUCCAGCA CUGAUGA X GAA AGGAAGUC
1800	ACUUCCUUU GCUGGAAA	UUUCCAGC CUGAUGA X GAA AAGGAAGU
1811	UGGAAAGUU AGUAAUCG	CGAUUACU CUGAUGA X GAA ACUUUCCA
1812	GGAAAGUUA GUAAUCGG	CCGAUUAC CUGAUGA X GAA AACUUUCC
1815	AAGUUAGUA AUCGGCUG	CAGCCGAU CUGAUGA X GAA ACUAACUU
1818	UUAGUAAUC GGCUGAUU	AAUCAGCC CUGAUGA X GAA AUUACUAA
1826	CGGCUGAUU CACGCAAU	AUUGCGUG CUGAUGA X GAA AUCAGCCG
1827	GGCUGAUUC ACGCAAUA	UAUUGCGU CUGAUGA X GAA AAUCAGCC
1835	CACGCAAUA AACUGCAA	UUGCAGUU CUGAUGA X GAA AUUGCGUG
1845	ACUGCAAUU GUGUAGUU	AACUACAC CUGAUGA X GAA AUUGCAGU
1850	AAUUGUGUA GUUUCUUA	UAAGAAAC CUGAUGA X GAA ACACAAUU
1853	UGUGUAGUU UCUUAAAU	AUUUAAGA CUGAUGA X GAA ACUACACA
1854	GUGUAGUUU CUUAAAUU	AAUUUAAG CUGAUGA X GAA AACUACAC
1855	UGUAGUUUC UUAAAUUU	AAAUUUAA CUGAUGA X GAA AAACUACA
1857	UAGUUUCUU AAAUUUGC	GCAAAUUU CUGAUGA X GAA AGAAACUA
1858	AGUUUCUUA AAUUUGCU	AGCAAAUU CUGAUGA X GAA AAGAAACU
1862	UCUUAAAUU UGCUAAUU	AAUUAGCA CUGAUGA X GAA AUUUAAGA
1863	CUUAAAUUU GCUAAUUC	GAAUUAGC CUGAUGA X GAA AAUUUAAG
1867	AAUUUGCUA AUUCUUAU	AUAAGAAU CUGAUGA X GAA AGCAAAUU
1870	UUGCUAAUU CUUAUUUG	CAAAUAAG CUGAUGA X GAA AUUAGCAA

Table V

Nt. Position	Substrate	Ribozyme
1871	UGCUAAUUC UUAUUUGA	UCAAAUAA CUGAUGA X GAA AAUUAGCA
1873	CUAAUUCUU AUUUGAUG	CAUCAAAU CUGAUGA X GAA AGAAUUAG
1874	UAAUUCUUA UUUGAUGA	UCAUCAAA CUGAUGA X GAA AAGAAUUA
1876	AUUCUUAUU UGAUGAUA	UAUCAUCA CUGAUGA X GAA AUAAGAAU
1877	UUCUUAUUU GAUGAUAU	AUAUCAUC CUGAUGA X GAA AAUAAGAA

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be $\geq$ 2 base-pairs.

Table VI: Potato Citrate Synthase Hairpin Ribozyme and Target Sequences

Nt. Position	Ribozyme	Substrate
15	CAAGUA AGAA GAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CAUCA GCC UACUUG
112	CUCGAG AGAA GAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GCUCC GCU CUCGAG
123	CUGUUG AGAA GCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCG GUC CAACAG
181	GAUCAA AGAA GGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUCCG GUC UUGAUC
285	CAUAUC AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACA GUU GAUAUG
354	UCCCUC AGAA GGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACCCU GAU GAGGGA
539	AUGAUG AGAA GGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCCCU GAU CAUCAU
579	UGGAUG AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UCACA GCU CAUCCA
596	GUAGCA AGAA GGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACCCA GUU UGCUAC
710	UGAGCA AGAA GAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAUCU GAU UGCUCA
735	AACAUA AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUGCU GCU UAUGUU
945	GUAAGG AGAA GACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UGUCU GAU CCUUAC
966	CAAAGC AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	UUGCU GCU GCUUUG
969	AUUCAA AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUGCU GCU UUGAAU
988	GAAGUG AGAA GGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AGCCG GAC CACUUC
1038	UUCUAC AGAA GAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAUCU GUU GUAGAA
1076	UCUUUC AGAA GCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GAGCA GUU GAAAGA
1214	AGUUGA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CCACU GUU UCAACU
1253	UGUAAG AGAA GGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUCCU GUU CUUACA
1356	AAAGAG AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AUACG GUC CUCUUU
1583	UUGAAG AGAA GACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	GGUCG GCC CUUCAA
1609	AGUUUG AGAA GAAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CUUCA GUU CAAACU
1720	UGCUGG AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	ACGCU GAU CCAGCA
1819	UGAAUC AGAA GAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	AAUCG GCU GAUUCA
1822	GCGUGA AGAA GCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	CGGCU GAU UCACCIC

Claims

1. An enzymatic nucleic acid molecule with RNA cleaving activity, wherein said nucleic acid molecule modulates the expression of a plant gene involved in the biosynthesis of alkaloid compounds.

2. The enzymatic nucleic acid molecule of claim 1, wherein said plant is a solanaceous plant.

3. The enzymatic nucleic acid molecule of claim 2, wherein said plant is selected from a group consisting of potato, tomato, pepper, eggplant and ditura.

4. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid is in a hammerhead configuration.

5. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid is in a hairpin configuration.

6. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid is in a hepatitis δ virus, group I intron, group II intron, VS nucleic acid or RNaseP nucleic acid configuration.

7. The enzymatic nucleic acid of claim 1, wherein said nucleic acid comprises between 12 and 100 bases complementary to RNA of said gene.

8. The enzymatic nucleic acid of claim 1, wherein said nucleic acid comprises between 14 and 24 bases complementary to RNA of said gene.

9. The enzymatic nucleic acid of claim 4, wherein said hammerhead comprises a stem II region of length greater than on equal to two base-pairs.

10. The enzymatic nucleic acid of claim 5, wherein said hairpin comprises a stem II region of length between three and seven base-pairs.

11. The enzymatic nucleic acid of claim 5, wherein said hairpin comprises a stem IV region of length greater than or equal to two base-pairs.

12. The enzymatic nucleic acid of claim 1, wherein said gene is solanidine UDP-glucose glucosyl-transferase.

13. The enzymatic nucleic acid molecule of claim 12, wherein said nucleic acid specifically cleaves any of sequences shown in Table III, wherein said nucleic acid is in a hammerhead configuration.

14. The enzymatic nucleic acid molecule of claim 12, wherein said nucleic acid specifically cleaves any of sequences shown in Table IV, wherein said nucleic acid is in a hairpin configuration.

15. The enzymatic nucleic acid molecule of any of claims 13 or 14, consisting essentially of one or more sequences selected from the group shown in Tables III and IV.

16. A plant cell comprising the enzymatic nucleic acid molecule of claim 1.

17. A transgenic plant and the progeny thereof, comprising the enzymatic nucleic acid molecule of claim 1.

18. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of claim 1, in a manner which allows expression and/or delivery of that enzymatic nucleic acid molecule within a plant cell.

19. An expression vector comprising nucleic acid encoding a plurality of enzymatic nucleic acid molecules of claim 1, in a manner which allows expression and/or delivery of said enzymatic nucleic acid molecules within a plant cell.

20. A plant cell comprising the expression vector of claim 18.

21. A plant cell comprising the expression vector of claim 19.

22. A transgenic plant and the progeny thereof, comprising the expression vector of claim 18.

23. A transgenic plant and the progeny thereof, comprising the expression vector of claim 19.

24. A method for modulating expression of an gene in a plant by administering to said plant the enzymatic nucleic acid molecule of claim 1.

25. The method of claim 24, wherein said plant is a potato plant.

26. The method of claim 24, wherein said gene is solanidine UDP-glucose glucosyl-transferase.

27. The expression vector of claim 18, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) a gene encoding at least one said enzymatic nucleic acid molecule; and

wherein said gene is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said enzymatic molecule within said plant cell.

28. The expression vector of claim 18, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) an open reading frame;

d) a gene encoding at least one said enzymatic nucleic acid molecule, wherein said gene is operably linked to the 3'-end of said open reading frame; and

wherein said gene is operably linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said enzymatic molecule within said plant cell.

29. The expression vector of claim 18, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) an intron;

d) a gene encoding at least one said enzymatic nucleic acid molecule; and

wherein said gene is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said enzymatic molecule within said plant cell.

30. The expression vector of claim 18, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) an intron;

d) an open reading frame;

e) a gene encoding at least one said enzymatic nucleic acid molecule, wherein said gene is operably linked to the 3'-end of said open reading frame; and

wherein said gene is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said enzymatic molecule within said plant cell.

31. A transgenic plant comprising nucleic acid molecule encoding for an enzymatic nucleic acid molecule with RNA cleaving activity, wherein said nucleic acid molecule modulates the expression of a gene involved in the biosynthesis of alkaloid in said plant .

32. The transgenic plant of Claim 31, wherein said gene is solanidine UDP-glucose glucosyl-transferase.

33. The transgenic plant of Claim 31, wherein the plant is transformed with Agrobacteriurn, bombarding with

DNA coated microprojectiles, whiskers, or electroporation.

34. The transgenic plant of Claim 33, wherein said bombarding with DNA coated microprojectiles is done with the gene gun.

35. The transgenic plant of Claim 31, wherein said plant contains a selectable marker selected from the group consisting of chlorosulfuron, hygromycin, bar gene, bromoxynil, and kanamycin and the like.

36. The transgenic plant of Claim 31, wherein said nucleic acid is operably linked to a promoter selected from the group consisting of octopine synthetase, the nopaline synthase, the manopine synthetase, cauliflower mosaic virus (35S); ribulose-1, 6-biphosphate (RUBP) carboxylase small subunit (ssu), the beta-conglycinin, the phaseolin promoter, napin, gamma zein, globulin, the ADH promoter, heat-shock, actin, and ubiquitin.

37. The transgenic plant of Claim 31, said enzymatic nucleic acid molecule is in a hammerhead, hairpin, hepatitis Δ virus, group I intron, group II intron, VS nucleic acid or RNaseP nucleic acid configuration

38. The transgenic plant of Claim 31, wherein said enzymatic nucleic acid with RNA cleaving activity encoded as a monomer.

39. The transgenic plant of Claim 31, wherein said enzymatic nucleic acid with RNA cleaving activity encoded as a multimer.

40. The transgenic plant of Claim 31, wherein the nucleic acids encoding for said enzymatic nucleic acid molecule with RNA cleaving activity is operably linked to the 3' end of an open reading frame.

41. An enzymatic nucleic acid molecule with RNA cleaving activity, wherein said nucleic acid molecule modulates the expression of a plant gene involved in the flower formation.

42. The enzymatic nucleic acid molecule of claim 41, wherein said plant is a potato plant.

43. The enzymatic nucleic acid molecule of claim 41, wherein said plant is selected from a group consisting of Lettuce, spinach, cabbage, brussel sprouts, arugula, kale, collards, chard, beet, turnip, sweet potato and turfgrass.

44. The enzymatic nucleic acid molecule of claim 41, wherein said nucleic acid is in a hammerhead configuration.

45. The enzymatic nucleic acid molecule of claim 41, wherein said nucleic acid is in a hairpin configuration.

46. The enzymatic nucleic acid molecule of claim 41, wherein said nucleic acid is in a hepatitis δ virus, group I intron, group II intron, VS nucleic acid or RNaseP nucleic acid configuration.

47. The enzymatic nucleic acid of claim 41, wherein said nucleic acid comprises between 12 and 100 bases complementary to RNA of said gene.

48. The enzymatic nucleic acid of claim 41, wherein said nucleic acid comprises between 14 and 24 bases complementary to RNA of said gene.

49. The enzymatic nucleic acid of claim 44, wherein said hammerhead comprises a stem II region of length greater than on equal to two base-pairs.

50. The enzymatic nucleic acid of claim 45, wherein said hairpin comprises a stem II region of length between three and seven base-pairs.

51. The enzymatic nucleic acid of claim 45, wherein said hairpin comprises a stem IV region of length greater than or equal to two base-pairs.

52. The enzymatic nucleic acid of claim 41, wherein said gene is citrate synthase.

53. The enzymatic nucleic acid molecule of claim 52, wherein said nucleic acid specifically cleaves any of sequences shown in Table V, wherein said nucleic acid is in a hammerhead configuration.

54. The enzymatic nucleic acid molecule of claim 52, wherein said nucleic acid specifically cleaves any of sequences shown in Table VI, wherein said nucleic acid is in a hairpin configuration.

55. The enzymatic nucleic acid molecule of any of claims 53 or 54, consisting essentially of one or more sequences selected from the group shown in Tables V and VI.

56. A plant cell comprising the enzymatic nucleic acid molecule of claim 41.

57. A transgenic plant and the progeny thereof, comprising the enzymatic nucleic acid molecule of claim 41.

58. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of claim 41, in a manner which allows expression and/or delivery of that enzymatic nucleic acid molecule within a plant cell.

59. An expression vector comprising nucleic acid encoding a plurality of enzymatic nucleic acid molecules of claim 41, in a manner which allows expression and/or delivery of said enzymatic nucleic acid molecules within a plant cell.

60. A plant cell comprising the expression vector of claim 58.

61. A plant cell comprising the expression vector of claim 59.

62. A transgenic plant and the progeny thereof, comprising the expression vector of claim 58.

63. A transgenic plant and the progeny thereof, comprising the expression vector of claim 59.

64. A method for modulating expression of an gene in a plant by administering to said plant the enzymatic nucleic acid molecule of claim 41.

65. The method of claim 64, wherein said plant is a potato plant.

66. The method of claim 64, wherein said gene is citrate synthase.

67. The expression vector of claim 58, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) a gene encoding at least one said enzymatic nucleic acid molecule; and

wherein said gene is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said enzymatic molecule within said plant cell.

68. The expression vector of claim 58, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) an open reading frame;

d) a gene encoding at least one said enzymatic nucleic acid molecule, wherein said gene is operably linked to the 3'-end of said open reading frame; and

wherein said gene is operably linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression

and/or delivery of said enzymatic molecule within said plant cell.

69. The expression vector of claim 58, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) an intron;

d) a gene encoding at least one said enzymatic nucleic acid molecule; and

wherein said gene is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said enzymatic molecule within said plant cell.

70. The expression vector of claim 58, wherein said vector comprises:

a) a transcription initiation region;

b) a transcription termination region;

c) an intron;

d) an open reading frame;

e) a gene encoding at least one said enzymatic nucleic acid molecule, wherein said gene is operably linked to the 3'-end of said open reading frame; and

wherein said gene is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said enzymatic molecule within said plant cell.

71. A transgenic plant comprising nucleic acid molecule encoding for an enzymatic nucleic acid molecule with RNA cleaving activity, wherein said nucleic acid

molecule modulates the expression of a gene involved in flower formation in said plant.

72. The transgenic plant of Claim 71, wherein said gene is citrate synthase.

73. The transgenic plant of Claim 71, wherein the plant is transformed with Agrobacteriurn, bombarding with DNA coated microprojectiles, whiskers, or electro-poration.

74. The transgenic plant of Claim 73, wherein said bombarding with DNA coated microprojectiles is done with the gene gun.

75. The transgenic plant of Claim 71, wherein said plant contains a selectable marker selected from the group consisting of chlorosulfuron, hygromycin, bar gene, bromoxynil, and kanamycin and the like.

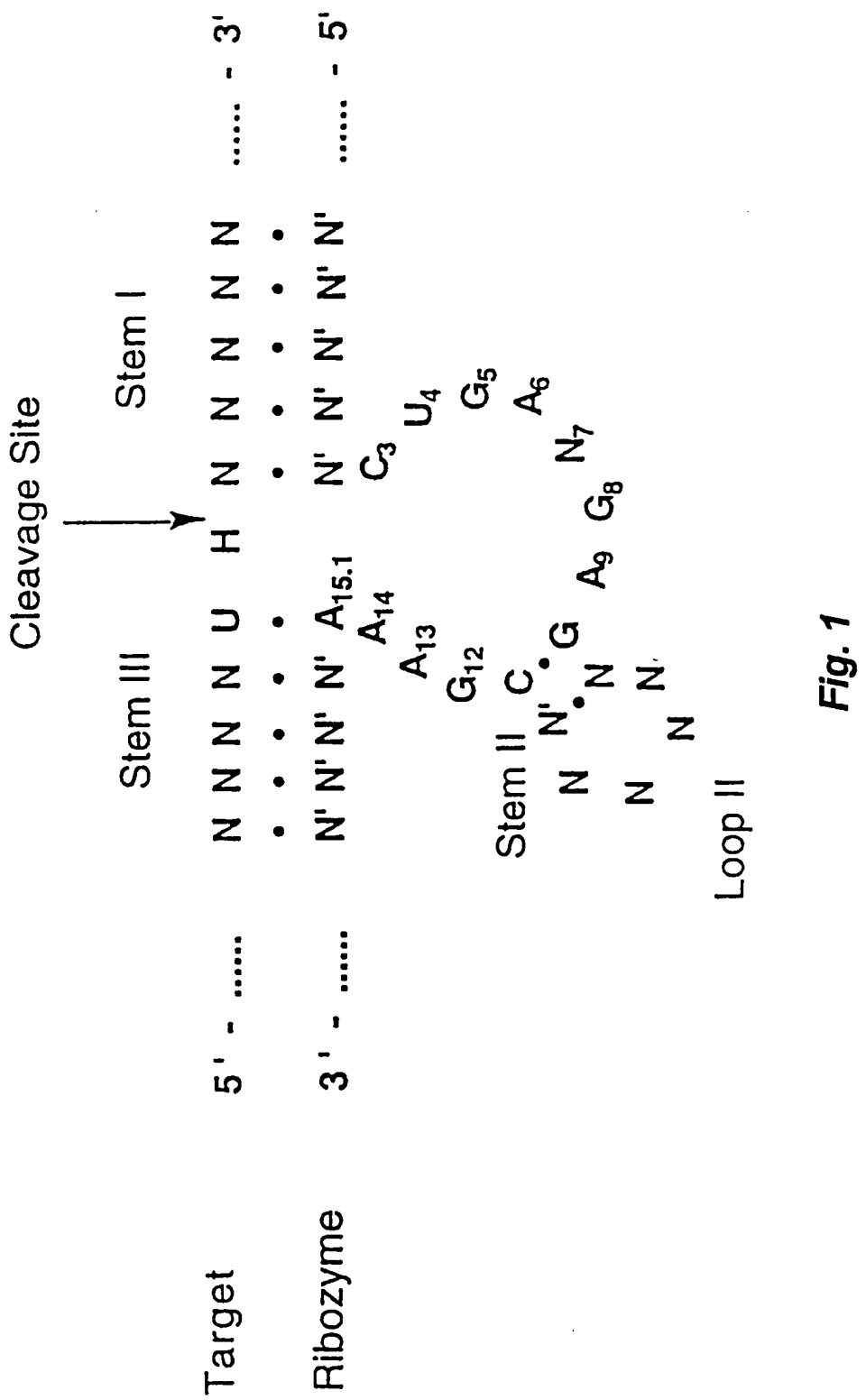
76. The transgenic plant of Claim 71, wherein said nucleic acid is operably linked to a promoter selected from the group consisting of octopine synthetase, the nopaline synthase, the manopine synthetase, cauliflower mosaic virus (35S); ribulose-1, 6-biphosphate (RUBP) carboxylase small subunit (ssu), the beta-conglycinin, the phaseolin promoter, napin, gamma zein, globulin, the ADH promoter, heat-shock, actin, and ubiquitin.

77. The transgenic plant of Claim 71, said enzymatic nucleic acid molecule is in a hammerhead, hairpin, hepatitis δ virus, group I intron, group II intron, VS nucleic acid or RNaseP nucleic acid configuration

78. The transgenic plant of Claim 71, wherein said enzymatic nucleic acid with RNA cleaving activity encoded as a monomer.

79. The transgenic plant of Claim 71, wherein said enzymatic nucleic acid with RNA cleaving activity encoded as a multimer.

80. The transgenic plant of Claim 71, wherein the nucleic acids encoding for said enzymatic nucleic acid molecule with RNA cleaving activity is operably linked to the 3' end of an open reading frame.


Cleavage Site
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Stem III
Stem II
Loop II
Target
Ribozyme
5' - .....
3' - .....
N N N N U H N N N N N .....- 3'
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A14
A13
G12
C3
U4
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G8
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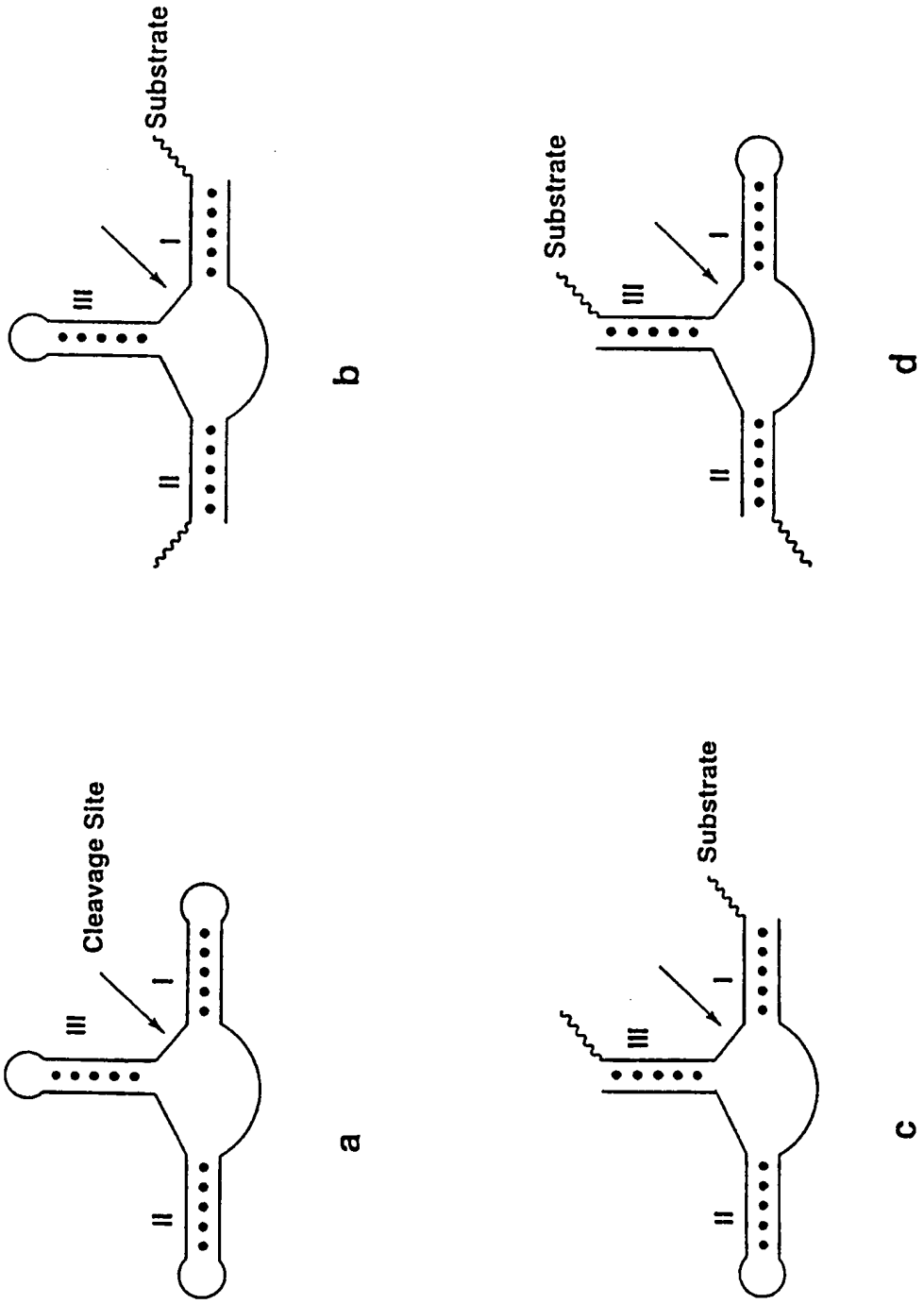
Fig. 1

Fig. 2


Cleavage Site
I
II
III
a
Substrate
I
II
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b
Substrate
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III
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Substrate
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III
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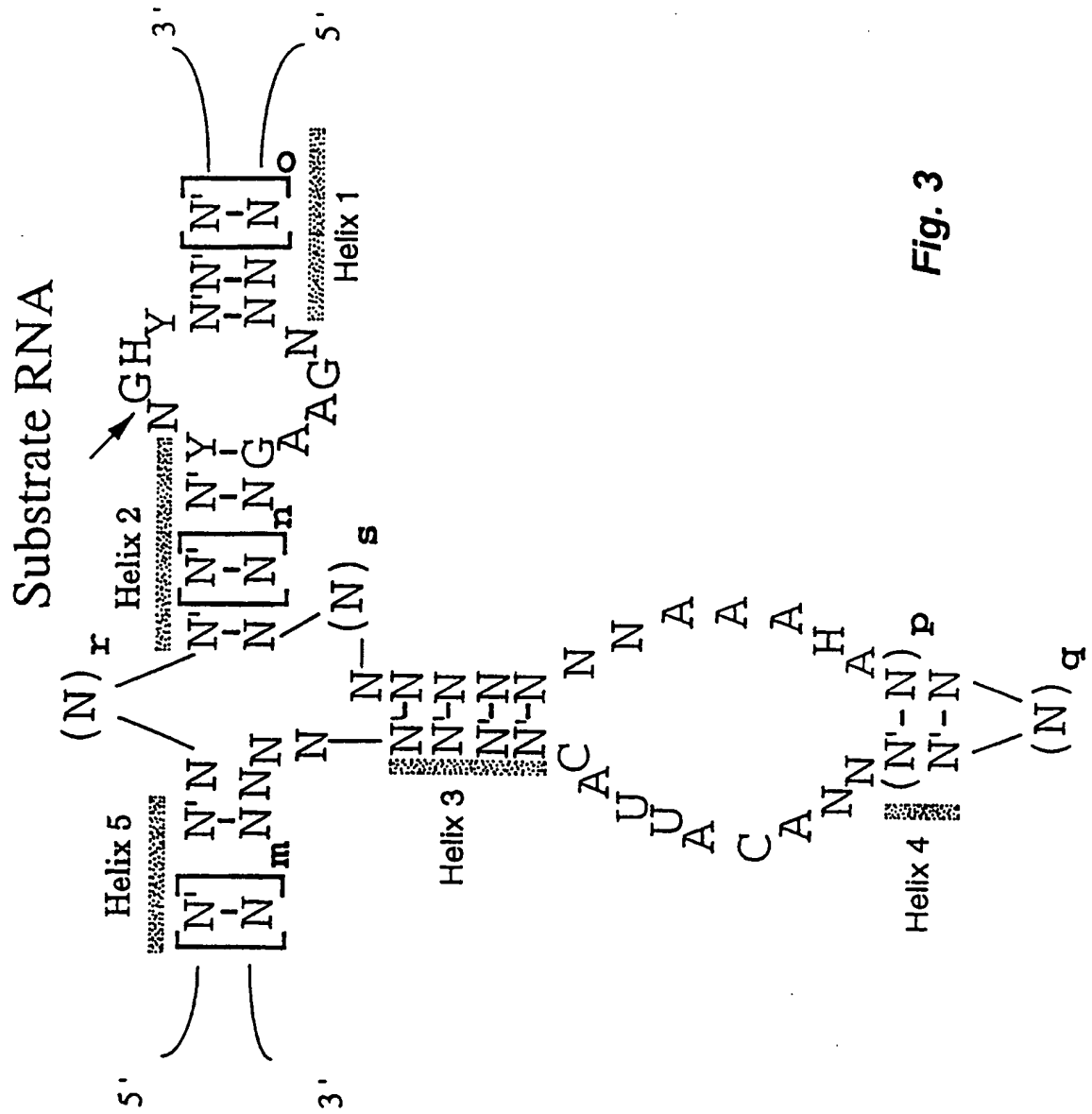

Fig. 3

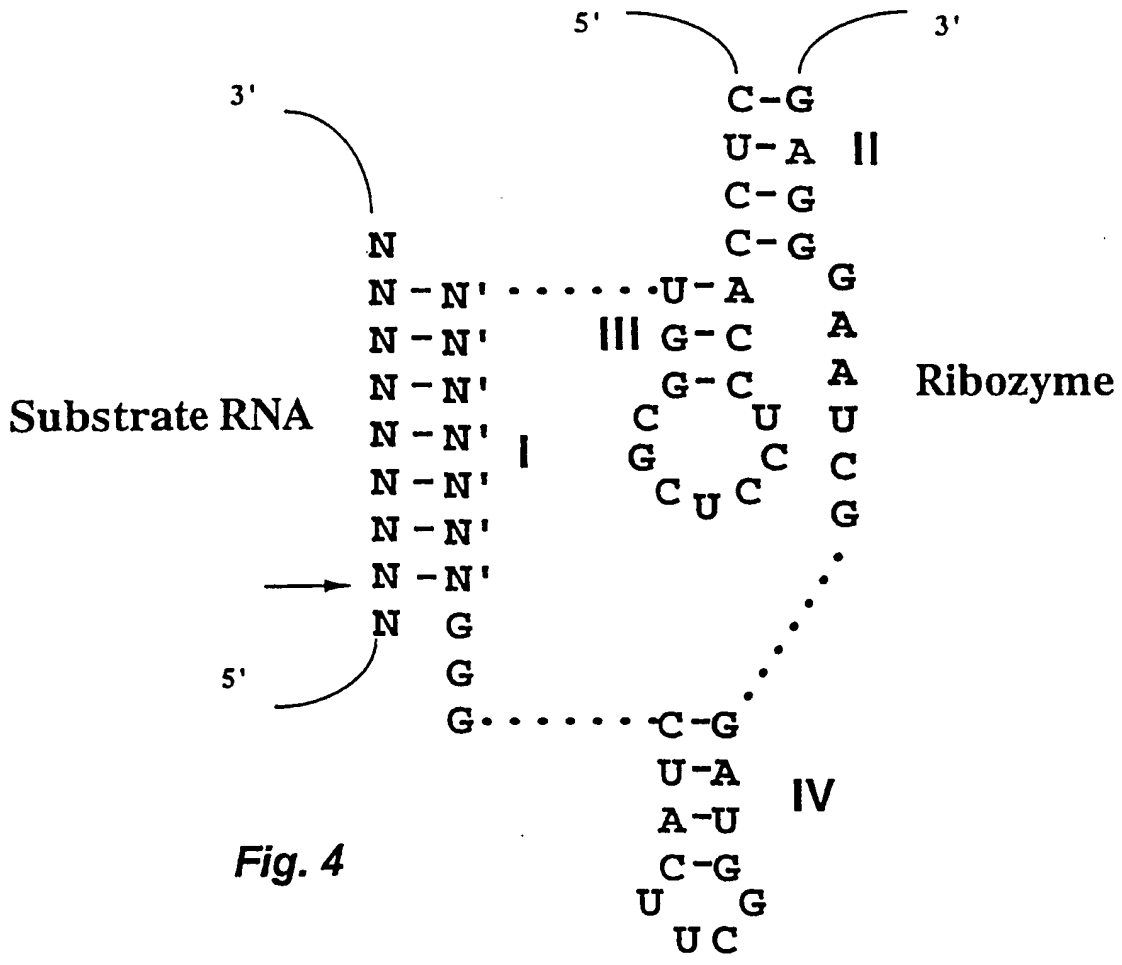

Fig. 4

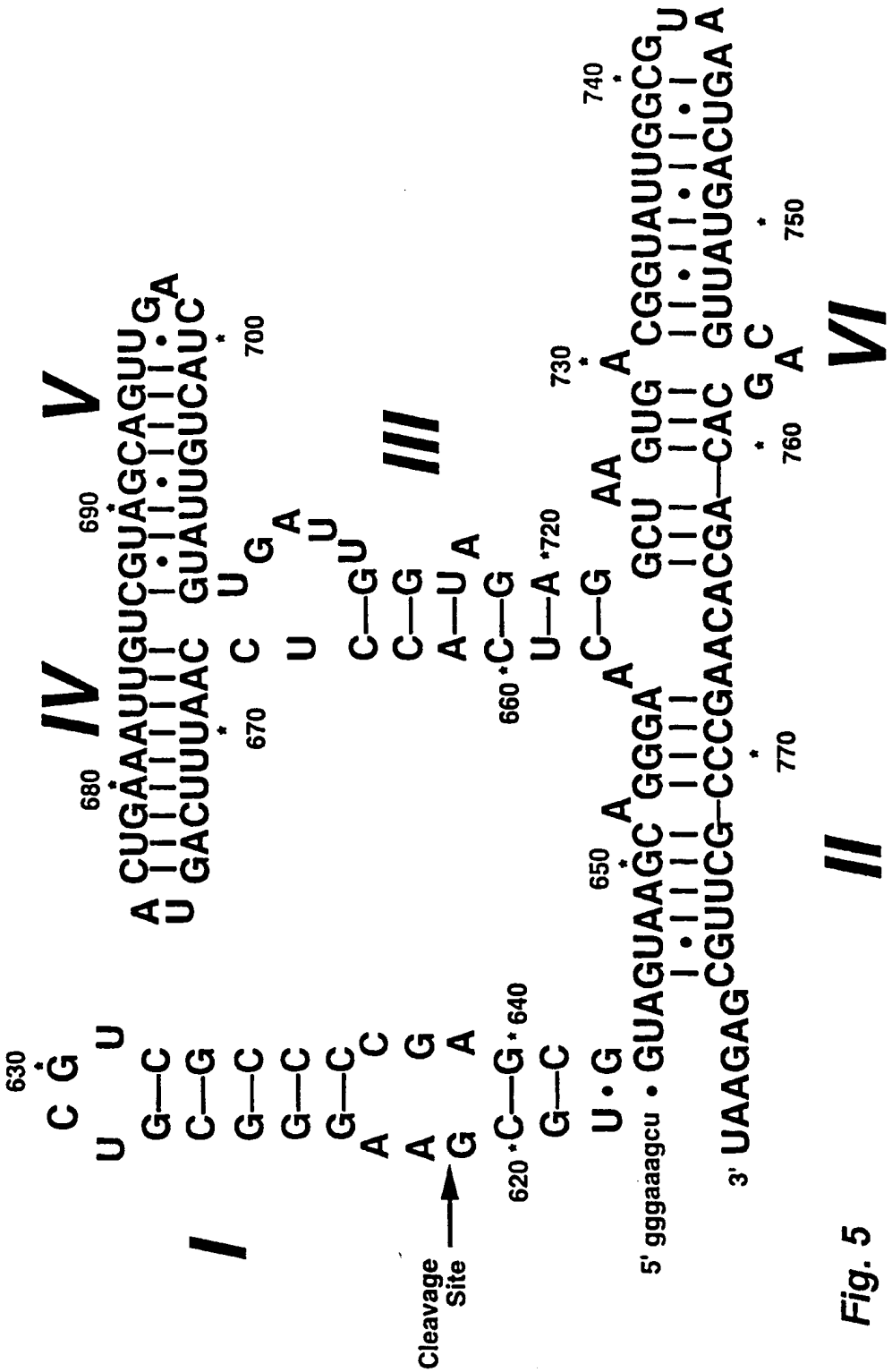

Fig. 5